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FORM PTO-1390 (Rev. 10-96) U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

ATTORNEY'S DOCKET NUMBER
001560-349

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

U.S. APPLICATION NO. (If known, see 37 CFR 1.5) 09/147947
Unassigned

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/JP 98/03324	24 July 1998	24 July 1997

TITLE OF INVENTION
NOVEL SERINE PROTEASE

APPLICANT(S) FOR DO/EO/US
Nobuo TSURUOKA, Kyoko YAMASHIRO and Nozomi YAMAGUCHI

Applicant herewith submits to the United States Designated/Elected Office (DO/EO/US) the following items and other information:

1. [x] This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.
2. [ ] This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 U.S.C. 371.
3. [x] This is an express request to begin national examination procedures (35 U.S.C. 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 U.S.C. 371(b) and the PCT Articles 22 and 39(1).
4. [ ] A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.
5. [x] A copy of the International Application as filed (35 U.S.C. 371(c)(2))
   - a. [ ] is transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [x] has been transmitted by the International Bureau.
   - c. [ ] is not required, as the application was filed in the United States Receiving Office (RO/US)
6. [x] A translation of the International Application into English (35 U.S.C. 371(c)(2)).
7. [x] Amendments to the claims of the International Application under PCT Article 19 (35 U.S.C. 371(c)(3))
   - a. [ ] are transmitted herewith (required only if not transmitted by the International Bureau).
   - b. [ ] have been transmitted by the International Bureau.
   - c. [ ] have not been made; however, the time limit for making such amendments has NOT expired.
   - d. [x] have not been made and will not be made.
8. [ ] A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).
9. [x] An oath or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).
10. [ ] A translation of the annexes to the International Preliminary Examination Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern other document(s) or information included:

11. [ ] An Information Disclosure Statement under 37 CFR 1.97 and 1.98.
12. [x] An assignment document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.
13. [x] A FIRST preliminary amendment.
    [ ] A SECOND or SUBSEQUENT preliminary amendment.
14. [ ] A substitute specification.
15. [ ] A change of power of attorney and/or address letter.
16. [x] Other items or information:

Notice Informing the Applicant of the Communication of the International Application to the Designated Offices and

International Search Report

U.S. APPLICATION NO. (If known, see 37 C.F.R. 1.50)	INTERNATIONAL APPLICATION NO.	ATTORNEY'S DOCKET NUMBER
Unassigned	PCT/JP 98/03324	001560-349

				CALCULATIONS	PTO USE ONLY
17. ☐ The following fees are submitted:					
Basic National Fee (37 CFR 1.492(a)(1)-(5)): Search Report has been prepared by the EPO or JPO .... $840.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) .... $670.00 No international preliminary examination fee paid to USPTO (37 CFR 1.482) but international search fee paid to USPTO (37 CFR 1.445(a)(2)) .... $760.00 Neither international preliminary examination fee (37 CFR 1.482) nor international search fee (37 CFR 1.445(a)(2)) paid to USPTO .... $970.00 International preliminary examination fee paid to USPTO (37 CFR 1.482) and all claims satisfied provisions of PCT Article 33(2)-(4) $96.00					
ENTER APPROPRIATE BASIC FEE AMOUNT =				$ 840.00	
Surcharge of **$130.00** for furnishing the oath or declaration later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(e)).				$	
Claims	Number Filed	Number Extra	Rate		
Total Claims	20 -20 =	0	X$18.00	$ 0.00	
Independent Claims	4 -3 =	1	X$78.00	$ 78.00	
Multiple dependent claim(s) (if applicable)			+ $260.00	$	
TOTAL OF ABOVE CALCULATIONS =				$ 918.00	
Reduction for 1/2 for filing by small entity, if applicable. Verified Small Entity statement must also be filed. (Note 37 CFR 1.9, 1.27, 1.28).				$ 0.00	-
SUBTOTAL =				$ 918.00	
Processing fee of **$130.00**for furnishing the English translation later than ☐ 20 ☐ 30 months from the earliest claimed priority date (37 CFR 1.492(f)). +				$ 0.00	
TOTAL NATIONAL FEE =				$ 0.00	
Fee for recording the enclosed assignment (37 CFR 1.21(h)). The assignment must be accompanied by an appropriate cover sheet (37 CFR 3.28, 3.31). $40.00 per property +				$ 40.00	
TOTAL FEES ENCLOSED =				$ 958.00	
				Amount to be: refunded	$
				charged	$

a. ☒ A check in the amount of $ 958.00 to cover the above fees is enclosed.

b. ☐ Please charge my Deposit Account No. 02-4800 in the amount of $______ to cover the above fees. A duplicate copy of this sheet is enclosed.

c. ☒ The Commissioner is hereby authorized to charge any additional fees which may be required, or credit any overpayment to Deposit Account No. 02-4800. A duplicate copy of this sheet is enclosed.

NOTE: Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.

SEND ALL CORRESPONDENCE TO:

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Donna M. Meuth
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NAME

36,607
REGISTRATION NUMBER

09/147947
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Patent
Attorney's Docket No. 001560-349

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re Patent Application of	
Nobuo TSURUOKA et al	Group Art Unit: Unassigned
Application No.: Unassigned Corresponding to PCT/JP 98/03324	Examiner: Unassigned
Filed: March 24, 1999	
For: NOVEL SERINE PROTEASE	

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, D.C. 20231

Sir:

Prior to examination on the merits, please amend the above identified application as follows:

IN THE SPECIFICATION:

In compliance with 37 C.F.R. § 1.823(a), please insert the attached copy of the "Sequence Listing" after page 28 and before the claims of the above-identified application.

IN THE CLAIMS:

Please amend claims 5, 6, 7, 10, and 11 as follows:

In claim 5, lines 2 and 3, please delete "in any one of the above-mentioned claims 1 to 4" and insert therefore --claim 1--.

In claim 6, lines 4 and 5, please delete "in any one of the above-mentioned claims 1 to 4" and insert therefore --claim 1--.

7. (Amended) An expression vector containing the DNA as claimed in [claims 5 or 6] claim 5.

In claim 10, lines 2 and 3, please delete "in any one of claims 1 to 4" and insert therefore --claim 1--.

11. (Amended) A process for screening physiologically active substance that uses the serine protease, domain or their partial peptides as claimed in claim 1 [any one of claims 1 to 4, or the DNA as claimed in claim 5 or 6].

Please add the following new claims:

--12. An expression vector containing the DNA as claimed in claim 6.

13. A process for screening physiologically active substance that uses the DNA as claimed in claim 5.

14. DNA which codes for the serine protease, domain or their partial peptides as claimed in claim 2.

15. DNA which codes for the serine protease, domain or their partial peptide as claimed in claim 3.

16. DNA which codes for the serine protease, domain or their partial peptide as claimed in claim 4.

17. An antibody whose antigen is the serine protease, domain or their partial peptide as claimed in claim 2.

18. An antibody whose antigen is the serine protease, domain or their partial peptide as claimed in claim 3.

19. An antibody whose antigen is the serine protease, domain or their partial peptide as claimed in claim 4.

20. A process for screening physiologically active substances that uses the DNA as claimed in claim 6.--

REMARKS

Entry of the foregoing and examination of the above-identified application is respectfully requested.

The paper copy of the Sequence Listing for the subject application, is by this amendment, added after page 28 and before the claims of the above-identified application. Please renumber the pages accordingly.

Claims 5, 6, 7, 10, and 11 have been amended to eliminate the multiple dependency of the claims. New claims 12-20 have been added, directed to preferred embodiments of the invention. These claims are supported by the original claims 1-11. No new matter has been added by these amendments.

Early and favorable action in the form of a Notice of Allowance is respectfully requested.

Application No. Unassigned
Attorney's Docket No. 001560-349

In the event that there are any questions relating to this amendment or the application in general, it would be appreciated if the Examiner would contact the undersigned attorney be telephone so that prosecution would be expedited.

Respectfully submitted,

BURNS, DOANE, SWECKER & MATHIS, L.L.P.

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Date: March 24, 1999

13PRTS

09/147947

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SPECIFICATION

NOVEL SERINE PROTEASE

Technical Field

The present invention relates to a novel serine protease, DNA coding therefor, a process for production of said serine protease, and a process for screening physiologically active substances using said serine protease or DNA coding therefor.

Background Art

Serine proteases are widely present in animals, plants and microorganisms, and are known to be involved in an extremely large number of biological reactions including food digestion, blood coagulation and fibrinolysis, complement activation, hormone production, ovulation and fertilization, phagocytosis, cell growth, development and differentiation, aging and cancer metastasis, particularly in higher animals (Neurath, H., Science, 224, 350-357, 1984).

In recent years, serine proteases have been confirmed to act as a physiologically important functional molecule in the central nervous system as well. For example, known serine proteases occurring in the brain include tissue plasminogen activator (Sappiro, A-D., Madani, R., Huarte, J., Belin, D., Kiss, J.Z., Wohlwent, A. and Vassalli, J-D., J. Clin. Invest., 92, 679-685, 1993), thrombin (Monard, D., Trends Neurosci., 11, 541-544, 1988), human trypsin IV (Wiegand, U., Corbach, S., Minn, A., Kang, J. and Müller-Hill, B., Gene, 136, 167-175, 1993), neuropsin (Chen, Z-L., Yoshida, S., Kato, K., Momota, Y., Suzuki, J., Tanaka, T., Ito, J., Nishino, H., Aimoto, S., Kiyama, H. and Shiosaka, S., J. Neurosci., 15(7), 5088-5097, 1995), and neurosin (Yamashiro, K., Tsuruoka, N., Kodama, S., Tsujimoto, M., Yamamura, Y., Tanaka, T., Nakazato, H. and

Yamaguchi, N., Biochim. Biophys. Acta, 1350, 11-14, 1997).

Not only are these serine proteases in the brain involved in the deployment of the neurite outgrowth of neurons, but they are also assumed to be involved in the synapse-formation process with target neurons (Liu, Y., Fields, R.D., Fitzgerald, S., Festoff, B.W. and Nelson, P.G., J. Neurobiol., 25, 325, 1994).

However, the physiological functions of these serine proteases in the brain are essentially unknown. In addition, although it is predicted that many other serine proteases exist that occur in the brain and are responsible for performing important physiological functions, the majority of these are still not identified.

On the other hand, certain types of serine protease proteins in the coagulation, fibrinolysis and complement system have the kringle domains, EGF-like structures, finger structures, γ-carboxyglutamic acid domains, apple domains and other structures on their N-terminus (Furie, B. and Furie, B.C., Cell, 53, 505-518, 1988). Examples of known serine protease proteins having some kringle domains include urokinase, plasminogen activator and plasminogen.

The kringle domains have the ability to bind with fibrin, heparin and lysine analogue (Scanu, A.M. and Edelstein, C., Biochimica. Biophysica. Acta, 1256, 1-12, 1995), and in the blood fibrinolysis system, plasminogen activator has been known to bind the precipitated fibrin by means of its kringle domains, following activation of nearby bound plasmin. Moreover, the angiogenesis inhibitory factor, angiostatin, has been identified to be the kringle domains in a plasminogen molecule (Cao, Y., Ji, R.W., Davidson, D., Scaller, J., Marti, D., Söhndel, S., McCance, S.G., O'Reilly, M.S., Llinás, M. and Folkman, J., J. Biol. Chem., 271, 29461-29467, 1996), and was shown for the first time to have physiological

activity as an independent Kringle domain, that provided the first demonstration of the physiological activity as kringle domains alone.

In addition, the existence of a series of protein groups including cyclophilin-C binding protein, speract receptor, complement factor I, CD5 and CD6 is known that have the scavenger receptor cysteine-rich (SRCR) domains observed in the macrophage scavenger receptor (Resnick, D., Pearson, A. and Krieger, M., Trends. Biochem. Sci., 19, 5-8, 1994).

In contrast to cyclophilin-C binding protein and complement factor I being secretory proteins, speract receptor, CD5 and CD6 are known to be membrane-bound proteins. Among these, a protein binding to membrane-bound protein CD6 was found to be the activated leukocyte adhesive molecule (ALCAM), and its binding site was localized to a SRCR domain structure of CD6 (Whitney, G.S., Starling, G.C., Bowen, M.A., Modrell, B., Siadak, A.W. and Aruffo, A.J., J. Biol. Chem., 270, 18187-18190, 1995).

Moreover, ALCAM, which is a ligand of CD6, is known to be expressed by activated lymphocytes and neurons, while CD6 is surmised to fulfill a certain regulatory function for maintaining homeostasis in the immune system and nervous system by means of the interaction with ALCAM.

In this manner, proteins composed of multi-domain structures not only have characteristic functions associated with each domain, but also are considered to function by having specific recognition functions interacting with each domain function.

Disclosure of the Invention

In consideration of the present circumstances as described above, the object of the present invention is to provide a novel serine protease, and a novel serine protease DNA that codes for it. Moreover, another object

of the present invention is to provide a process for producing a large amount of said protease using said DNA, and a process for screening physiologically active substances using said serine protease or DNA that codes for it.

As a result of repeated earnest research, the inventors of the present invention isolated cDNA that codes for a novel functional protein by screening cDNA having a characteristic 5' translation region using a region preserved well in cDNA for the probe that codes for serine protease occurring in the brain, thereby leading to completion of the present invention.

Thus, the present invention provides (1) a serine protease or its partial peptide containing an amino acid sequence identical to serine protease indicated in Figs. 7 to 12 (SEQ ID NO: 6), an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted, or an amino acid sequence in which at least one amino acid is added to the identical amino acid sequence or an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted.

Moreover, the present invention provides (2) a serine protease domain or its partial peptide containing an amino acid sequence identical to a serine protease domain comprising the amino acid sequence from amino acid no. 578 to 822 indicated in Figs. 7 to 12 (SEQ ID NO: 6), an amino acid sequence in which a portion of the identical sequence is deleted or substituted, or an amino acid sequence in which at least one amino acid is added to the identical amino acid sequence or an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted.

Moreover, the present invention provides (3) a kringle domain or its partial peptide containing an amino acid sequence identical to a kringle domain comprising the amino acid sequence from amino acid no. 40 to 112

indicated in Figs. 7 to 12 (SEQ ID NO: 6), an amino acid sequence in which a portion of the identical sequence is deleted or substituted, or an amino acid sequence in which at least one amino acid is added to the identical amino acid sequence or an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted.

Moreover, the present invention provides (4) a scavenger receptor cysteine-rich (SRCR) domain or its partial peptide containing an amino acid sequence identical to an SRCR domain comprising the amino acid sequence from amino acid no. 117 to 217, from amino acid no. 227 to 327, from amino acid No. 334 to 433, or from amino acid No. 447 to 547 indicated in Figs. 7 to 12 (SEQ ID NO: 6), an amino acid sequence in which a portion of the identical sequence is deleted or substituted, or an amino acid sequence in which at least one amino acid is added to the identical amino acid sequence or an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted.

Moreover, the present invention provides (5) DNA which codes for the serine protease, domain or their partial peptides as set forth in any one of the above-mentioned (1) to (4).

Moreover, the present invention provides (6) DNA which codes for a peptide having serine protease, domain or their partial peptide activity, and is hybridizable with DNA that codes for the serine protease, domain or their partial peptides as set forth in any one of the above-mentioned (1) to (4) under stringent conditions.

Moreover, the present invention provides (7) an expression vector containing the DNA as set forth in the above-mentioned (5) or (6).

Moreover, the present invention provides (8) a host transformed by the expression vector as set forth in the above-mentioned (7).

Moreover, the present invention provides (9) a

process for preparing of serine protease, domain or their partial peptides comprising culturing or breeding the host as set forth in the above-mentioned (8), and harvesting serine protease, domain or their partial peptides.

Moreover, the present invention provides (10) an antibody whose antigen is the serine protease, domain or their partial peptides as set forth in any one of the above-mentioned (1) to (4).

Moreover, the present invention provides (11) a process for screening physiologically active substances that uses the serine protease, domain or their partial peptides as set forth in any one of the above-mentioned (1) to (4), or the DNA as set forth in the above-mentioned (5) or (6).

Brief Description of the Drawings

Fig. 1 indicates a portion of the nucleotide sequence of cDNA that codes for mouse serine protease, and its corresponding amino acid sequence.

Fig. 2 indicates a portion of the nucleotide sequence of cDNA that codes for mouse serine protease, and its corresponding amino acid sequence.

Fig. 3 indicates a portion of the nucleotide sequence of cDNA that codes for mouse serine protease, and its corresponding amino acid sequence.

Fig. 4 indicates a portion of the nucleotide sequence of cDNA that codes for mouse serine protease, and its corresponding amino acid sequence.

Fig. 5 indicates a portion of the nucleotide sequence of cDNA that codes for mouse serine protease, and its corresponding amino acid sequence.

Fig. 6 indicates a portion of the nucleotide sequence of cDNA that codes for mouse serine protease, and its corresponding amino acid sequence.

Fig. 7 indicates a portion of the nucleotide sequence of cDNA that codes for human serine protease,

and its corresponding amino acid sequence.

Fig. 8 indicates a portion of the nucleotide sequence of cDNA that codes for human serine protease, and its corresponding amino acid sequence.

Fig. 9 indicates a portion of the nucleotide sequence of cDNA that codes for human serine protease, and its corresponding amino acid sequence.

Fig. 10 indicates a portion of the nucleotide sequence of cDNA that codes for human serine protease, and its corresponding amino acid sequence.

Fig. 11 indicates a portion of the nucleotide sequence of cDNA that codes for human serine protease, and its corresponding amino acid sequence.

Fig. 12 indicates a portion of the nucleotide sequence of cDNA that codes for human serine protease, and its corresponding amino acid sequence.

Fig. 13 is an electrophoresis diagram indicating the results of Northern blotting that shows transcription of serine protease gene in various mouse organs.

Mode for Carrying Out the Invention

Cloning of cDNA coding for mouse serine protease was performed by first preparing a cDNA library from mouse brain mRNA isolated and prepared in accordance with conventional methods, and then performing PCR using the cDNA library and PCR primers designed and prepared based on a serine protease motif. Using the resulting PCR product as a probe, clones were screened having a long 5' translation region and expected to code for a novel functional protein.

As a result, the inventors of the present invention succeeded in isolating a 2.7 kb cDNA named mouse BSSP-3. As a result of investigating the resulting cDNA sequence in accordance with conventional methods, mouse BSSP-3 cDNA was determined to code for a novel functional protein that contains not only a serine protease domain, but also a kringle domain and scavenger receptor

cysteine-rich domains. The isolated mouse BSSP-3 cDNA coded for one Kringle domain, three scavenger receptor cysteine-rich domains, and one serine protease domain. A specific example is described in Example 1.

Next, when expression of mouse BSSP-3 mRNA was confirmed in various mouse organs and various sites of mouse brain using the entire length of the isolated mouse BSSP-3 cDNA as a probe, with respect to expression in various mouse organs, strong expression was observed particularly in the brain, while expression was also observed in the lung and kidney. In addition, with respect to various sites of mouse brain, strong expression was observed in the cerebrum and brain stem, and expression was also observed in the medulla oblongata. The size was only about 2.7 kb in all cases. Of the various sites in the brain that were examined, expression of mouse BSSP-3 mRNA was not observed in the cerebellum. A specific example is described in Example 2. Based on these findings, mouse BSSP-3 mRNA was confirmed to actually be expressed in mouse organs.

Moreover, as a result of screening the human brain cDNA library using mouse BSSP-3 cDNA as a probe, human BSSP-3 cDNA was able to be successfully isolated. As a result, the inventors of the present invention clearly showed that human BSSP-3 cDNA clearly differs from that which would be predicted from the primary structure of mouse BSSP-3 cDNA, and was determined to code for one kringle domain, four scavenger receptor cysteine-rich domains, and one serine protease domain. A specific example is described in Example 3. Moreover, when the inventors of the present invention expressed human BSSP-3 cDNA coding for serine protease mature protein in COS-1 cells, it was clearly determined to be a functional protein having enzyme activity. A specific example is described in Example 4.

Based on the above results, in terms of its primary structure, the mouse and human BSSP-3 cDNAs isolated here

encode a novel functional protein that not only contain a novel serine protease domain, a novel kringle domain and novel scavenger receptor cysteine-rich domains, but also is functional proteins in which the serine protease domain has enzyme activity.

Not only is it clear that the novel functional protein in the present invention has complex functions due to its primary structure, but it also plays a certain role in the physiological function of the brain through the complex functions. Thus, the mouse BSSP-3 cDNA and novel functional protein encoded by the mouse BSSP-3 cDNA of the present invention provide useful means for pathological analysis of various types of mouse disease models. In addition, the human BSSP-3 cDNA and novel functional protein encoded by human BSSP-3 cDNA of the present invention also provide useful means for screening therapeutic agents for various types of diseases based on useful information for disease treatment obtained through pathological analysis. Moreover, they can also be applied to the development of therapeutic drugs for actual human diseases.

Examples of these treatment methods include supplementary treatment by administration of the recombinant protein and the gene-expression promotion or inhibition therapy by the sense or antisense method. Moreover, each of the domain structures of the novel functional protein can also function independently. Thus, molecules that exhibit interaction with each domain structure can be identified after expressing each domain structure separately. In addition, by investigating the involvement in disease of the identified molecule group, supplementary treatment by administration of the recombinant protein and gene-expression promotion or inhibition therapy by the sense or antisense method can be applied.

The following provides an explanation of the present invention based on its examples.

Although the present invention discloses the nucleotide sequence indicated in Figs. 1 to 6 (SEQ ID NO: 3) and Figs. 7 to 12 (SEQ ID NO: 5) as nucleotide sequences of DNA that code for novel serine proteases, the serine protease DNAs of the present invention are not limited to them. Once the amino acid sequence of naturally-occurring serine protease is determined, various nucleotide sequences that code for the same amino acid sequence can be designed based on codon degeneration and prepared. In this case, it is preferable to use codons that are used at high frequency in a host to be used.

In order to obtain DNA that codes for naturally-occurring serine protease of the present invention, although cDNA can be obtained in the manner described in the examples, it is not limited to this. Namely, once a single nucleotide sequence that codes for the amino acid sequence of naturally-occurring serine protease is determined, DNA coding for naturally-occurring serine protease can be cloned as cDNA by a strategy that differs from the strategy specifically disclosed in the present invention. Moreover, it can also be cloned from a genome of cells that produce it.

For example, the above-mentioned DNA can be cloned by the polymerase chain reaction (PCR) method using a DNA (nucleotide) primers as shown in Example 1.

The DNA of the present invention also codes for a protein or glycoprotein having serine protease activity, and includes DNA that hybridizes with the nucleotide sequence of Figs. 1 to 6 (SEQ ID NO: 3) or Figs. 7 to 12 (SEQ ID NO: 5). In addition, typical hybridization methods are well known among persons with ordinary skill in the art (examples of which include Experimental Medicine, special edition, Yodosha Publishing, "Biotechnology Experimental Method Series - Gene Engineering General Collection", Vol. 1.5, No. 11, pp. 24-60, 1987), and measurement of activity is also well

known among persons with ordinary skill in the art.

In the case of cloning from a genome, the various primer nucleotides or probe nucleotides used in the examples can be used as probes for selecting genome DNA fragments. In addition, other probes can also be used that are designed based on the nucleotide sequence described in Figs. 1 to 6 (SEQ ID NO: 3) or Figs. 7 to 12 (SEQ ID NO: 5). Typical methods for cloning a target DNA from the genome are also well known among persons with ordinary skill in the art (Current Protocols in Molecular Biology, John Wiley & Sons, publisher, Chapters 5 and 6).

The DNA that codes for naturally-occurring serine protease of the present invention can also be prepared by chemical synthesis. DNA chemical synthesis can be easily performed by a person with ordinary skill in the art by using an automated DNA synthesizer such as the 396 DNA/RNA synthesizer of Applied Biosystems. Thus, a person with ordinary skill in the art can easily synthesize DNA of the nucleotide sequence indicated in Figs. 1 to 6 (SEQ ID NO: 3) or Figs. 7 to 12 (SEQ ID NO: 5).

A DNA that codes for naturally-occurring serine protease according to codons that differ from the native codons can also be prepared by chemical synthesis as mentioned above, and can also be obtained in accordance with conventional methods such as site-directed mutagenesis using a mutagenic primer with DNA or RNA having the nucleotide sequence indicated in Figs. 1 to 6 (SEQ ID NO: 3) or Figs. 7 to 12 (SEQ ID NO: 5) as a template (see, for example, Current Protocols in Molecular Biology, John Wiley & Sons, publisher, Chapter 8).

In this manner, once the amino acid sequence is determined, various variant forms of serine protease can be designed and produced, including polypeptides in which one or more amino acids are added to the naturally-occurring amino acid sequence while maintaining serine

protease activity, polypeptides in which one or more amino acids are deleted from the above-mentioned naturally-occurring amino acid sequence while maintaining serine protease activity, polypeptides in which one or more amino acids in the above-mentioned naturally-occurring amino acid sequence are substituted with another amino acids while maintaining serine protease activity, and modified polypeptides in which the above-mentioned amino acid addition modification, amino acid deletion modification and amino acid substitution modification are combined, while maintaining serine protease activity.

Although there are no particular restrictions on the numbers of amino acids in the above-mentioned modification including amino acid addition, deletion or substitution modification, with respect to addition, the number of amino acids is dependent on the number of amino acids of known functional protein, e.g. maltose-binding protein, used to form a hybrid protein with the serine protease of the present invention for the purpose of extraction, purification or stabilization or on that of proteins having various physiological activities or the signal peptide added to the present serine protease. Namely, the number of amino acids to be modified is determined depending on the purpose of said modification, and for example, 1 to 50, and preferably 1 to 10, are added.

In addition, with respect to deletion, the number of amino acids that are deleted is designed and determined so as to maintain serine protease activity, and is, for example, 1 to 30, and preferably 1 to 20, or may be, for example, the number of amino acids in a region other than the active region of the present serine protease. Moreover, with respect to substitution, the number of amino acids that are substituted is designed and determined so as to maintain the serine protease activity, and is, for example, 1 to 10, and preferably 1

to 5.

In addition, the present invention provides a serine protease domain comprising the amino acid sequence from amino acid No. 517 to 761 or No. 578 to 822 indicated in Figs. 1 to 6 (SEQ ID NO: 4) or Figs. 7 to 12 (SEQ ID NO: 6), respectively, a kringle domain comprising the amino acid sequence from amino acid No. 85 to 157 or from No. 40 to 112 indicated in Figs. 1 to 6 (SEQ ID NO: 4) or Figs. 7 to 12 (SEQ ID NO: 6), respectively, or scavenger receptor cysteine-rich (SRCR) domains comprising the amino acid sequence from amino acid No. 166 to 266, from No. 273 to 372, from No. 386 to 486, from No. 117 to 217, from No. 227 to 327, from No. 334 to 433 or from No. 447 to 547 indicated in Figs. 1 to 6 (SEQ ID NO: 4) or Figs. 7 to 12 (SEQ ID NO: 6), respectively. Production of these domains can be performed by the method described later, a peptide synthesis method which itself is known, or by cleaving said serine protease by a suitable protease. In addition, modified domains that maintain the activity of the domains of the present invention or DNA that code for them can also be similarly produced.

When DNA of the serine protease or domain of the present invention is obtained in the manner described above, a recombinant serine protease or domain can be produced by ordinary gene recombination using the DNA for serine protease or domain. Namely, DNA coding for the serine protease or domain of the present invention is inserted into a suitable expression vector, said expression vector is introduced into suitable host cells, said host cells are cultured, and the target serine protease or domain is recovered from the resulting culture (cells or medium).

The serine protease or domain of the present invention may be obtained in a biochemically or chemically modified form, such as acylation of its N-terminal, including formylation, acetylation or other C_{1-6} acylation or deletion. The secretion efficiency and

expression level of the expression system may be improved by addition or modification of a signal sequence, or selection of host. Examples of addition and modification of signal sequence include a method in which a gene coding for a signal peptide of another structural peptide is ligated upstream of the 5'-end of the structural gene of the serine protease or domain of the present invention through a gene coding for a cleavable partial peptide. Specific examples of this include methods using the signal sequence of the trypsin gene and using a gene coding for an enterokinase recognition sequence, as described in Example 4.

Prokaryotic or eukaryotic organisms can be used for the host. Examples of prokaryotes that can be used include bacteria, and particularly *Escherichia coli* and the genus *Bacillus* such as *Bacillus subtilis*. Examples of eukaryotes that can be used include yeast such as the genus *Saccharomyces* such as *Saccharomyces cerevisiae*, and other eukaryotic microorganisms, insect cells such as armyworm cells (*Spodoptera frugiperda*), cabbage looper cells (*Trichoplusia ni*) and silkworm cells (*Bombyx mori*), and animal cells such as human cells, monkey cells and mice cells, specific examples of which include COS-1 cells, Vero cells, CHO cells, L cells, myeloma cells, C127 cells, BALB/c3T3 cells and Sp-2/O cells. Organisms themselves can also be used in the present invention, including insects such as cabbage looper and silkworm.

Examples of expression vectors that can be used include plasmids, phages, phagemids and viruses (Baculovirus (insects), Vaccinia virus (animal cells)) etc. A promoter in an expression vector is selected dependent on the host cells, and examples of bacterial promoters that are used include lac promoter and trp promoter, while examples of yeast promoters that are used include adhI promoter and pqk promoter.

In addition, examples of insect promoters include Baculovirus polyhedrin promoter, while examples of animal

cell promoters include Simian Virus 40 early or late promoter, CMV promoter, HSV-TK promoter and SRα promoter. In addition, it is preferable to use an expression vector containing an enhancer, splicing signal, poly A addition signal, selective marker (such as dihydrofolate reductase gene (methotrexate-resistant) or neo gene (G418-resistant) in addition to those indicated above. Furthermore, in case of using an enhancer, SV40 enhancer, for example, is inserted upstream or downstream from the gene.

Transformation of host with an expression vector can be performed in accordance with conventional methods well known in the art, and these methods are described in, for example, Current Protocols in Molecular Biology, John Wiley & Sons, publisher. Culturing of the transformant can also be performed in accordance with conventional methods. Purification of serine protease or domain from the culture can be performed in accordance with conventional methods for isolation and purification of proteins, examples of which include ultrafiltration and various types of column chromatography such as chromatography using Sepharose.

Since the serine protease or domain of the present invention thus obtained is a functional protein, it provides a useful means for pathological analysis, allows screening of physiologically active substances using this protein, and is useful in research searching for therapeutic agents for various diseases. As a specific example of a screening method, screening for example of serine protease inhibitor, can be performed in the same manner as Example 4 by measuring a physiological activity of a tested sample, for example, a naturally-occurring component such as a peptide, protein, non-peptide compound, synthetic compound or fermentation product or compounds obtained from the culture supernatant of various cells, or artificial component an such as various types of synthetic compounds.

In addition, the above-mentioned measurement of physiological activity, measurement of binding affinity and so forth using the serine protease, domain or their partial peptides of the present invention or hosts transformed by DNA coding for the above-mentioned serine protease, domain or their partial peptides or its cell membrane fraction are also preferable embodiments of the screening method of the present invention.

In addition, DNA coding for the serine protease, domain or their partial peptides of the present invention is provided as a useful means of supplementary therapy by administration of the recombinant protein, the gene-expression promotion or inhibition therapy using the sense or antisense method, and elucidation of physiological functions within the body, and is also used for screening of new drugs based on the resulting information.

Moreover, the serine protease, domain or their partial peptides of the present invention, or DNA coding for them can be provided as a kit in a form that can be used when carrying out the above-mentioned screening methods.

Examples of partial peptides include peptide fragments comprising specific region of serin protase of the present invention such as peptide fragments present in the vicinity of a serine residue of an active site as well as peptide fragments that can be antibody recognition sites specific for the serine protease or domain of the present invention. Furthermore, production of said partial peptides can be performed by the methods previously described with respect to the serine protease or domain of the present invention, a peptide synthesis method which is itself known, or by cleaving said serine protease or domain with a suitable protease.

In addition, the above-mentioned cell membrane fraction refers to the fraction containing a large amount of cell membrane obtained after culturing host cells that

allow expression of DNA coding for the serine protease, domain or its partial peptides of the present invention, under conditions that allow expression, and disrupting the resulting host cells containing serine protease, domain or its partial peptides by a method which is itself known.

A screening method for physiologically active substances using the serine protease, domain or its partial peptides of the present invention is performed by screening samples to be tested using the serine protease, domain or its partial peptides of the present invention, DNA coding for them, host cells containing said serine protease, domain or its partial peptides, or its cell membrane fraction. As a specific example of such method, screening is performed by measuring activity or measuring binding affinity using a substrate of the serine protease, domain or its partial peptides of the present invention, examples of which include a synthetic substrate such as a color-development substrate, or a substrate labeled with a radioisotope.

Furthermore, in the case of using host cells containing serine protease, domain or their partial peptides, the cells can be used after fixing with a known method (with glutaraldehyde, formaldehyde, etc.). In addition, in the case of using DNA coding for said serine protease, domain or their partial peptides, a technique for evaluating promotion or inhibition of gene expression can be performed using a reporter gene such as luciferase gene.

Examples

Example 1. Cloning of Novel Serine Protease Motif cDNA for Use as a Probe

(1) PCR Using a Serine Protease Conservative Region

Preparation of mouse brain mRNA was performed using an RGT-T-primed first-strand kit (Pharmacia) in accordance with the attached instructions. 2 μl (1 μg)

of oligo-dT primer was added to 5 μl (about 6 μg) of the resulting mRNA and heated for 10 minutes at 70°C followed by cooling rapidly on ice.

4 μl of 5x first strand buffer (250 mM Tris-HCl, pH 8.3, 375 mM KCl, 15 mM $MgCl_2$), 1 μl of 10 mM dNTP, 2 μl of 0.1 M DTT, diethylpyrocarbonate (DEPC)-treated distilled water and 5 μl (1000 U) of Super Script IIRT were added to the denatured mRNA, and allowed to react for 1 hour at 37°C. PCR was then performed using the serine protease conservative region and the resulting first strand cDNA as the template.

Oligomer KY185 (5'-GTG CTC ACN GCN GCB CAY TG-3') shown in SEQ ID NO: 1 and synthesized based on the amino acid conservative region in the vicinity of an active residue (His) (N-Val-Leu-Thr-Ala-Ala-His-Cys), and oligomer KY189 (3'-CCV CTR AGD CCN CCN GGC GA-5') shown in SEQ ID NO: 2 and synthesized based on the amino acid preservation region in the vicinity of an active residue (Ser) (N-Gly-Asp-Ser-Gly-Gly-Pro-Leu), were used as primers. After performing PCR using Taq DNA polymerase (Amersham), the PCR reaction solution was subcloned to pCRII vector (Invitrogen).

(2) Isolation and Purification of Mouse Brain mRNA for Screening

Preparation of mouse brain mRNA was performed using the Fast Track mRNA Isolation Kit (Invitrogen) in accordance with the attached instructions. Namely, 15 ml of lysis buffer was added to the entire extracted mouse brain and homogenized immediately with a teflonhomogenizer. After passing the homogenized tissue through a 21 gauge injection needle three times using a syringe, it was placed in a 50 ml centrifuge tube and incubated for 1 hour in a water bath at 45°C.

After incubation, the homogenized tissue was centrifuged for 5 minutes at 4000 x g, and the resulting supernatant was transferred to another 50 ml centrifuge

tube. After adding 950 µl of 5 M NaCl solution, the solution was again passed through a 21 gauge injection needle three times using a syringe. Next, 1 tablet of oligo(dT) cellulose was added to the solution and after allowing to swell for 2 minutes, the solution was slowly rocked for 1 hour. One hour later, the solution was centrifuged for 5 minutes at 2,000 x g and after aspirating off the supernatant, the precipitate was suspended in 20 ml of binding buffer followed by washing the centrifuged residue in 10 ml of binding buffer.

Next, the precipitate was washed three times with 10 ml of low salt washing solution. After the final washing, the oligo(dT) cellulose was suspended in 800 µl of low salt washing solution, placed in a spin column, and centrifugal washing was repeated three times for 10 seconds at 5000 x g. After washing, 200 µl of elution buffer were added followed by repeating centrifugation for 10 seconds at 5000 x g twice to obtain 400 µl of mRNA solution. mRNA was recovered from the mRNA solution by ethanol precipitation in accordance with conventional methods, and dissolved in 20 µl of DEPC-treated distilled water.

(3) Screening from a cDNA Library

<Step 1> Synthesis of cDNA

2 µl (1 µg) of oligo dT NotI primer was added to 5 µl (about 6 µg) of the mRNA obtained in Example 1, part (2), and heated for 10 minutes at 70°C followed by cooling rapidly on ice. 4 µl of 5x first strand buffer (250 mM Tris-HCl pH 8.3, 375 mM KCl, 15 mM $MgCl_2$), 1 µl of 10 mM dNTP, 2 µl of 0.1 M DTT, DEPC-treated distilled water and 5 µl (1000 U) of Super Script IIRT were added to this denatured mRNA and allowed to react for 1 hour at 37°C.

Next, 91 μl of DEPC-treated distilled water, 30 μl of 5x second strand buffer (100 mM Tris-HCl pH 6.9, 450 mM KCl, 23 mM $MgCl_2$, 0.75 mM β-NAD+, 50 mM $(NH_4)_2SO_4$), 3 μl of 10 mM dNTP, 1 μl (10 U) of E. coli DNA ligase, 4 μl (40 U) of E. coli DNA polymerase and 1 μl (2 U) of E. coli RNAase H were added to this reaction solution, and after reacting for 2 hours at 16°C, 2 μl (10 U) of T4 DNA polymerase was added and allowed to react for 5 minutes at 16°C.

Moreover, 10 μl of 0.5 M EDTA was added to this solution and after mixing, 150 μl of phenol:chloroform:isoamyl alcohol (25:24:1) was added. After stirring, the solution was centrifuged for 5 minutes at 15,000 rpm and the supernatant was recovered. 10 μl of 5 M KOAc and 400 μl of ethanol were added to the resulting supernatant followed by stirring and centrifuging for 10 minutes at 15,000 rpm. The precipitate obtained by centrifugation was washed with 500 μl of 70% ethanol and after gently air drying, was dissolved in 25 μl of DEPC-treated distilled water.

<Step 2> Addition of EcoRI Adapter

10 μl of 5 x T4 DNA linking buffer (250 mM Tris-HCl pH 7.6, 50 mM $MgCl_2$, 5 mM ATP, 5 mM DTT, 25% (W/v) PEG 8000), 10 μl (10 μg) of EcoRI adapter solution and 5 μl (5 U) of T4 DNA ligase were added to 25 μl of the double strand cDNA obtained in the previous step. After reacting for 16 hours at 16°C, 50 μl of phenol:chloroform:isoamyl alcohol (25:24:1) was added, stirred and centrifuged for 5 minutes at 15,000 rpm followed by recovery of the supernatant. 5 μl of 5 M KOAc and 125 μl of ethanol were added to the recovered supernatant and stirred. After cooling for 20 minutes at

-80°C, the supernatant was centrifuged for 10 minutes at 15,000 rpm. The precipitate resulting from centrifugation was washed with 200 μl of 70% ethanol and after gently air-drying, was dissolved in 40 μl of DEPC-treated distilled water.

<Step 3> Ligation with λgt 10

1 μl (50 ng) of λgt 10 (EcoRI fragment) was added to 3 ml of size-fractionated cDNA solution followed by the addition of 11 μl of DEPC-treated distilled water, 4 μl of 5 x T4 DNA linking buffer and 1 μl of 5 x T4 DNA ligase and allowing to react for 3 hours at room temperature. After extracting with phenol:chloroform:isoamyl alcohol (25:24:1), adding 5 μl (5 μg) of yeast tRNA, 5 μl of 5 M KOAc and 125 μl of ethanol and stirring, the mixture was cooled for 20 minutes at -80°C and centrifuged for 10 minutes at 15,000 rpm. The precipitate resulting from centrifugation was washed with 200 μl of 70% ethanol and after gently air-drying, was dissolved in 5 μl of TE (10 mM Tris-HCl pH 8.0, 1 mM EDTA).

<Step 4> Packaging

The ligated cDNA obtained in step 3 was packaged using Gigapack Packaging Extracts (Stratagene). Namely, after adding 10 μl of Freeze-thaw Extract contained in the kit to 1 μl of 0.1 μg/μl ligated cDNA, 15 μl of Sonic Extract contained in the kit was immediately added and mixed well. After allowing to stand for 2 hours at room temperature, 500 μl of phage dilution buffer (100 mM NaCl, 10 mM $MgSO_4$, 50 mM Tris-HCl pH 7.5, 0.01% gelatin) was added followed by addition of 20 μl of chloroform. After mixing well, the mixture was centrifuged for 5 minutes at the room temperature at 15,000 rpm and the supernatant was recovered to obtain a phage solution.

According to conventional methods, it was used to infect the host E. coli after titrating the phase solution.

<Step 5> Library Screening

The DNA fragment obtained in Example 1, part (1) was labeled with α-^{32}P dCTP using the BcaBest DNA labeling kit (Takara) to prepare a probe. A cDNA library comprising approximately 400,000 clones obtained in the previous step was screened using this probe. As a result, the longest clone of the inserted DNA fragment, pUC18/mBSSP-3/1-1 was obtained from the approximately 400,000 clones.

The total length of the pUC18/mBSSP-3/1-1 cDNA was 2,597 base pairs, and consisted of a 5' non-translation region of 244 base pairs, a translation region of 2283 base pairs, and a 3' non-translation region of 70 base pairs. The translation region was determined to code for a novel functional protein containing not only a serine protease domain (amino acid No. 517 to 761), but also a kringle domain (amino acid No. 85 to 157) and three scavenger receptor cysteine-rich domains (amino acid No. 166 to 266: domain 1, amino acid No. 273 to 372: domain 2, and amino acid No. 386 to 486: domain 3). The nucleotide sequence and corresponding amino acid sequence of pUC18/mBSSP-3/1-1 cDNA are shown in Figs. 1 to 6 (SEQ ID NO: 3).

Example 2. Examination of Expression Site of mBSSP-3 by Northern Blotting

Mouse brain total RNA was prepared using Trizol reagent (Life Technology) in accordance with the attached instructions. Namely, after extracting mouse cerebrum, brain stem, cerebellum and medulla oblongata, the tissues were immediately homogenized with a Polytron (Kinematica), and the tissues were lysed by addition of 10 volumes (approx. 3 ml) of Trizol reagent relative to tissue volume. Moreover, 600 μl of chloroform was added, followed by mixing and centrifuging for 15 minutes at 4°C and 15,000 rpm. After centrifugation, the aqueous phase

was recovered and 1500 µl of isopropanol was added to the recovered aqueous phase, followed by mixing and centrifuging for 30 minutes at 4°C and 15,000 rpm.

After dissolving the resulting total RNA precipitate of each site of mouse brain in 400 µl of DEPC-treated distilled water, it was blotted onto a membrane filter in accordance with conventional methods. Next, pUC18/mBSSP-3/1-1 was digested with restriction enzyme EcoRI, followed by isolation and purification of an approximately 2.7 kbp DNA fragment to prepare a probe by labeling with α-^{32}P dCTP using the above-mentioned method.

After hybridizing this probe overnight at 55°C with the membrane filters blotted with the total RNA prepared from each of the mouse brain sites described above, and with membrane filters blotted with commercially available mRNA prepared from various organs (Clontech), each of the membrane filters was washed for 20 minutes at room temperature with 2 x SSC containing 1% SDS (150 mM NaCl, 15 mM sodium citrate), and then washed twice for 30 minutes at 65°C after changing to 0.1 x SSC and 0.1% SDS. The membrane filters were then exposed for 30 minutes on a BAS2000 imaging plate (Fuji Photo Film).

The results are shown in Fig. 13. With respect to expression in each organ, expression was confirmed in the brain, lung and kidney. With respect to each site of the brain, strong expression was observed in the cerebrum and brain stem. Although weak expression was also observed in the medulla oblongata, expression was not observed in the cerebellum. The expressed size was only about 2.7 kbp in all cases.

<u>Example 3 Cloning of Human BSSP-3 cDNA</u>

Human brain cDNA library was purchased from Clontech. Mouse BSSP-3 cDNA fragment was fluorescent labeled using glutaraldehyde to prepare a probe.

pUC18/hBSSP-3 was obtained as a result of screening the human brain cDNA library comprising approximately 400,000 clones using this probe.

The translation region of pUC18/hBSSP-3 cDNA was determined to code for a functional protein containing not only a serine protease domain (amino acid No. 578 to 822), but also a kringle domain (amino acid No. 40 to 112) and four scavenger receptor cysteine-rich domains (amino acid No. 117 to 217: domain 1, amino acid No. 227 to 327: domain 2, amino acid No. 334 to 433: domain 3, and amino acid No. 447 to 547: domain 4) in the same manner as mouse BSSP-3 cDNA.

However, it was clearly different from that predicted from the primary structure of mouse BSSP-3 cDNA. In contrast to mouse BSSP-3 having three scavenger receptor cysteine-rich domains, human BSSP-3 was determined to have four such domains. The nucleotide sequence and corresponding amino acid sequence of pUC18/hBSSP-3 are shown in Figs. 7 to 12 (SEQ ID NO: 5).
pUC18/hBSSP-3 are shown in Figs. 7 to 12 (SEQ ID NO: 5).

<u>Example 4. Measurement of Enzyme Activity of Novel Serine Protease Mature Protein Coded by Human BSSP-3 cDNA</u>

(1) Construction of Expression Plasmid

pUC18/hBSSP-3 DNA fragment and pdKCR vector DNA fragment were ligated in accordance with conventional methods, *E. coli* JM109 was transformed, and the resulting colonies were analyzed by PCR to obtain the target serine protease hBSSP-3 expression plasmid pdKCR/hBSSP-3.

Next, primers were designed by amplifying genes coding for the signal sequence following the starting methionine of trypsin II and enterokinase recognition sequence so that EcoRI restriction enzyme recognition site was added upstream from the 5' side and BspMI restriction enzyme recognition site was added downstream from the 3' side. Using these primers, PCR was performed using pCR/Trypsin II plasmid for a template, and the product was digested with restriction enzymes (EcoRI and

BspMI), followed by isolation and purification of an approximately 75 bp DNA fragment. Similarly, using a primer designed so that a BspMI restriction enzyme recognition site is added upstream from DNA coding for a mature protein of human BSSP-3, PCR was performed using pdKCR/hBSSP-3 for the template, followed by digestion of the product with restriction enzymes (BspMI and Bpu1102I) and isolation and purification of the DNA fragment.

Next, a resulting DNA fragment coding for trypsin II signal sequence and enterokinase recognition site, and a DNA fragment coding for human BSSP-3 mature protein were ligated into pdKCR/hBSSP-3 vector predigested with restriction enzymes (BspMI and Bpu1102I) in accordance with conventional methods, followed by transformation of *E. coli* JM109. Transformed colonies containing the target chimeric DNA were confirmed by PCR to obtain the expression plasmid (pdKCR/Trp-hBSSP-3).

(2) Expression in COS-1 Cells

Chimeric gene DNA prepared in Example 4, part (1) was transfected into COS-1 cells using lipofectin (Life Technologies). Namely, 5 x 10^5 COS-1 cells were grown in Dalvecco's minimum essential medium (DMEM, Nissui Pharmaceutical) containing 10% fetal bovine serum in 10 cm diameter culture dishes (Corning, 430167). On the following day, after rinsing the cells with 5 ml of Opti-MEM medium (Life Technologies), 5 ml of fresh Opti-MEM medium was added, followed by culturing for 2 hours at 37°C.

After culturing, a mixture of 1 µg of the above-mentioned plasmid and 5 µg of lipofectin was added to each dish, followed by culturing for 5 hours at 37°C. After culturing, 5 ml of Opti-MEM medium was added to make a total volume of 10 ml, followed by additional culturing for 72 hours at 37°C. After culturing, the culture supernatant was collected by centrifugation to

prepare samples for measurement of enzyme activity. In addition, culture supernatant was prepared for use as a control by transfecting only expression plasmid pdKCR into COS-1 cells.

(3) Measurement of Enzyme Activity

The enzyme activity in the culture supernatant obtained in Example 4, part (2) was measured. Namely, 5 μl of enterokinase (10 mg/ml, Biozyme Laboratories) was mixed with 45 μl of culture supernatant of COS-1 cells and allowed to react for 2 hours at 37°C. Next, 50 μl of 0.2 mM substrate solution prepared by dissolving synthetic substrate Boc-Phe-Ser-Arg-MCA (Peptide Research) in DMSO and diluted with 0.1 M Tris-HCl, pH 8.0 was added and allowed to react for 16 hours at 4°C. After reacting, fluorescence was measured at an excitation wavelength of 485 nm and fluorescent wavelength of 535 nm. As a result, enzyme activity was only observed when culture supernatant of COS-1 cells that expressed Trp-hBSSP-3 were digested with enterokinase.

Based on the above results, the serine protease domain of human BSSP-3 was determined to be a functional protein having enzyme activity.

Effect of the Invention

The inventors of the present invention isolated mouse BSSP-3 cDNA from mouse brain cDNA library, that codes for a novel functional protein containing not only a novel serine protease domain, but also a novel kringle domain and novel scavenger receptor cysteine-rich domains. The isolated mouse BSSP-3 cDNA coded for 1 Kringle domain, 3 scavenger receptor cysteine-rich domains and 1 serine protease domain. In addition, as a result of examining the expression sites of the isolated mouse BSSP-3 mRNA, the inventors of the present invention determined that mouse BSSP-3 mRNA is strongly expressed

in the brain, and particularly strongly in the cerebrum and brain stem.

Next, the inventors of the present invention succeeded at isolating human BSSP-3 cDNA from a human brain cDNA library using mouse BSSP-3 cDNA as a probe. As a result, the inventors of the present invention determined that human BSSP-3 cDNA is clearly different from that predicted from the primary structure of mouse BSSP-3 cDNA, in that it was determined to code for 1 kringle domain, 4 scavenger receptor cysteine-rich domains, and 1 serine protease domain.

Moreover, the inventors of the present invention determined that, when human BSSP-3 cDNA coding for serine protease mature protein was expressed in COS-1 cells, the expression product is a functional protein having enzyme activity. Not only was the novel functional protein in the present invention determined to have complex functions in terms of its primary structure, but that it plays a constant role in the physiological functions in the brain through the complex functions. Thus, the mouse BSSP-3 cDNA and novel functional protein encoded by the mouse BSSP-3 cDNA of the present invention provide useful means of pathological analysis of various types of mouse disease models.

In addition, the human BSSP-3 cDNA and novel functional protein encoded by the human BSSP-3 cDNA of the present invention provide means for screening therapeutic agents for various diseases based on the useful information for disease treatment obtained through the above pathological analysis. Moreover, they can also be applied to actual development of therapeutic drugs for human diseases. Examples of such treatment methods include supplementary therapy by administration of the recombinant protein and gene-expression promotion or inhibition therapy using the sense or antisense method.

Moreover, the structure of each domain of the novel functional proteins can also function independently.

Thus, molecules that demonstrate interaction with each domain structure can be specified after separately expressing each domain structure. In addition, supplementary therapy by administration of the recombinant protein and the gene-expression promotion or inhibition therapy using the sense or antisense method can be performed by investigating the involvement of the specified molecular group in a disease.

CLAIMS

1. A serine protease or its partial peptide comprising an amino acid sequence identical to serine protease indicated in SEQ ID NO: 6, an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted, or an amino acid sequence in which at least one amino acid is added to the identical amino acid sequence or an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted.

2. A serine protease domain or its partial peptide comprising an amino acid sequence identical to a serine protease domain comprising the amino acid sequence from amino acid No. 578 to 822 indicated in SEQ ID NO: 6, an amino acid sequence in which a portion of the identical sequence is deleted or substituted, or an amino acid sequence in which at least one acid is added to the identical amino acid sequence or an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted.

3. A kringle domain or its partial peptide comprising an amino acid sequence identical to a kringle domain comprising the amino acid sequence from amino acid No. 40 to 112 indicated in SEQ ID NO: 6, an amino acid sequence in which a portion of the identical sequence is deleted or substituted, or an amino acid sequence in which at least one amino acid is added to the identical amino acid sequence or an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted.

4. A scavenger receptor cysteine-rich (SRCR) domain or its partial peptide comprising an amino acid sequence identical to an SRCR domain comprising the amino acid sequence from amino acid No. 117 to 217, from amino acid No. 227 to 327, from amino acid No. 334 to 433, or from amino acid No. 447 to 547 indicated in SEQ ID NO: 6, an amino acid sequence in which a portion of the

identical sequence is deleted or substituted, or an amino acid sequence in which at least one amino acid is added to the identical amino acid sequence or an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted.

5. DNA which codes for the serine protease, domain or their partial peptides as claimed in any one of the above-mentioned claims 1 to 4.

6. DNA which codes for a peptide having serine protease, domain or their partial peptide activity, and is hybridizable with DNA that codes for the serine protease, domain or their partial peptides as claimed in any one of the above-mentioned claims 1 to 4 under stringent conditions.

7. An expression vector containing the DNA as claimed in claims 5 or 6.

8. A host transformed by the expression vector as claimed in claim 7.

9. A process for preparing serine protease, domain or their partial peptides comprising culturing or breeding a host as claimed in claim 8, and recovering serine protease, domain or their partial peptides.

10. An antibody whose antigen is the serine protease, domain or their partial peptides as claimed in any one of claims 1 to 4.

11. A process for screening physiologically active substances that uses the serine protease, domain or their partial peptides as claimed in any one of claims 1 to 4, or the DNA as claimed in claims 5 or 6.

ABSTRACT

The present invention discloses a serine protease or its partial peptide containing an amino acid sequence identical to serine protease indicated in SEQ ID NO: 6, an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted, or an amino acid sequence in which at least one amino acid is added to the identical amino acid sequence or an amino acid sequence in which a portion of the identical amino acid sequence is deleted or substituted.

Fig.1

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CGAGGGTGGGGTGGAGGTCGGACTCCGGGCTACAGAGCTCCTGGCGCTCATCGCCTCTGG    60
CTCCAGCCTTTGCTTCGCGGGGCTGACCCTTTGGGTCCCGGTGTGATCCTCCAGCTGCCC   120
CGGGGGCTGGGACAGCAGGGCGGCGGCGCGAGCGTGGGAGGGGGCTCTAGGACTCTGCCG   180
GCCCCGCCCCGCCCCCTCCGCGGGACCCGGAGCCCAGCATGGACCACACTCGGCGCCGC   240
AGCC                                                          244
  ATGGCGCTCGCCCGCTGCGTGCTGGCTGTGATTTTAGGGGCACTGTCTGTAGTGGCC     301
  MetAlaLeuAlaArgCysValLeuAlaValIleLeuGlyAlaLeuSerValValAla    19

CGCGCTGATCCGGTCTCGCGCTCTCCCCTTCACCGCCCGCATCCGTCCCCACCGCGTTCC   361
ArgAlaAspProValSerArgSerProLeuHisArgProHisProSerProProArgSer    39

CAACACGCGCACTACCTTCCCAGCTCGCGGCGGCCACCCAGGACCCCGCGCTTCCCGCTC   421
GlnHisAlaHisTyrLeuProSerSerArgArgProProArgThrProArgPheProLeu    59

CCGCTGCGGATCCCCGCTGCCCAGCGCCCGCAGGTCCTCAGCACCGGGCACACGCCCCCG   481
ProLeuArgIleProAlaAlaGlnArgProGlnValLeuSerThrGlyHisThrProPro    79

ACGATTCCACGCCGCTGCGGGGCAGGAGAGTCGTGGGGCAATGCCACCAACCTCGGCGTC   541
ThrIleProArgArgCysGlyAlaGlyGluSerTrpGlyAsnAlaThrAsnLeuGlyVal    99

CCGTGTCTACACTGGGACGAGGTGCCGCCCTTCCTGGAGCGGTCGCCCCCGGCCAGTTGG   601
ProCysLeuHisTrpAspGluValProProPheLeuGluArgSerProProAlaSerTrp   119
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Fig.2

GCTGAGCTGCGAGGGCAGCCGCACAACTTCTGCCGGAGCCCGGATGGCTCGGGCAGACCT	661
AlaGluLeuArgGlyGlnProHisAsnPheCysArgSerProAspGlySerGlyArgPro	139
TGGTGCTTCTATCGGAATGCCCAGGGCAAAGTAGACTGGGGCTACTGCGATTGTGGTCAA	721
TrpCysPheTyrArgAsnAlaGlnGlyLysValAspTrpGlyTyrCysAspCysGlyGln	159
GGCCCGGCGTTGCCCGTCATTCGCCTTGTTGGTGGGAACAGTGGGCATGAAGGTCGAGTG	781
GlyProAlaLeuProValIleArgLeuValGlyGlyAsnSerGlyHisGluGlyArgVal	179
GAGCTGTACCACGCTGGCCAGTGGGGGACCATCTGTGACGACCAATGGGACAATGCAGAC	841
GluLeuTyrHisAlaGlyGlnTrpGlyThrIleCysAspAspGlnTrpAspAsnAlaAsp	199
GCAGACGTCATCTGTAGGCAGCTGGGGCTCAGTGGCATTGCCAAAGCATGGCATCAGGCA	901
AlaAspValIleCysArgGlnLeuGlyLeuSerGlyIleAlaLysAlaTrpHisGlnAla	219
CATTTTGGGGAAGGATCTGGCCCAATATTGTTGGATGAAGTACGCTGCACCGGAAACGAG	961
HisPheGlyGluGlySerGlyProIleLeuLeuAspGluValArgCysThrGlyAsnGlu	239
CTGTCAATTGAGCAATGTCCAAAGAGTTCCTGGGGCGAACATAACTGTGGCCATAAAGAA	1021
LeuSerIleGluGlnCysProLysSerSerTrpGlyGluHisAsnCysGlyHisLysGlu	259

Fig.3

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GATGCTGGAGTGTCTTGTGTTCCTCTAACAGATGGTGTCATCAGACTGGCAGGAGGAAAA   1081
AspAlaGlyValSerCysValProLeuThrAspGlyValIleArgLeuAlaGlyGlyLys    279

AGTACCCATGAAGGTCGCCTGGAGGTCTACTACAAGGGGCAGTGGGGACAGTCTGTGAT    1141
SerThrHisGluGlyArgLeuGluValTyrTyrLysGlyGlnTrpGlyThrValCysAsp    299

GATGGCTGGACTGAGATGAACACATACGTGGCTTGTCGACTGCTGGGATTTAAATACGGC   1201
AspGlyTrpThrGluMetAsnThrTyrValAlaCysArgLeuLeuGlyPheLysTyrGly    319

AAACAGTCCTCTGTGAACCATTTTGATGGCAGCAACAGGCCCATATGGCTGGATGACGTC   1261
LysGlnSerSerValAsnHisPheAspGlySerAsnArgProIleTrpLeuAspAspVal    339

AGCTGCTCAGGAAAAGAAGTCAGCTTCATTCAGTGTTCCAGGAGACAGTGGGGAAGGCAT   1321
SerCysSerGlyLysGluValSerPheIleGlnCysSerArgArgGlnTrpGlyArgHis    359

GACTGCAGCCATAGAGAAGATGTGGGCCTCACCTGCTATCCTGACAGCGATGGACATAGG   1381
AspCysSerHisArgGluAspValGlyLeuThrCysTyrProAspSerAspGlyHisArg    379

CTTTCTCCAGGTTTTCCCATCAGACTAGTGGATGGAGAGAATAAGAAGGAAGGACGAGTG   1441
LeuSerProGlyPheProIleArgLeuValAspGlyGluAsnLysLysGluGlyArgVal    399
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Fig.4

```
GAGGTTTTTGTCAATGGCCAATGGGGAACAATCTGCGATGACGGATGGACCGATAAGCAT   1501
GluValPheValAsnGlyGlnTrpGlyThrIleCysAspAspGlyTrpThrAspLysHis    419

GCAGCTGTGATCTGCCGGCAGCTTGGCTATAAGGGTCCTGCCAGAGCAAGGACTATGGCT   1561
AlaAlaValIleCysArgGlnLeuGlyTyrLysGlyProAlaArgAlaArgThrMetAla    439

TATTTTGGGGAAGGAAAAGGCCCCATCCACATGGATAATGTGAAGTGCACAGGAAATGAG   1621
TyrPheGlyGluGlyLysGlyProIleHisMetAspAsnValLysCysThrGlyAsnGlu    459

AAGGCCCTGGCTGACTGTGTCAAACAAGACATTGGAAGGCACAACTGCCGCCACAGTGAG   1681
LysAlaLeuAlaAspCysValLysGlnAspIleGlyArgHisAsnCysArgHisSerGlu    479

GATGCAGGAGTCATCTGTGACTATTTAGAGAAGAAAGCATCAAGTAGTGGTAATAAAGAG   1741
AspAlaGlyValIleCysAspTyrLeuGluLysLysAlaSerSerSerGlyAsnLysGlu    499

ATGCTCTCATCTGGATGTGGACTGAGGTTACTGCACCGTCGGCAGAAACGGATCATTGGT   1801
MetLeuSerSerGlyCysGlyLeuArgLeuLeuHisArgArgGlnLysArgIleIleGly    519

GGGAACAATTCTTTAAGGGGTGCCTGGCCTTGGCAGGCTTCCCTCAGGCTGAGGTCGGCC   1861
GlyAsnAsnSerLeuArgGlyAlaTrpProTrpGlnAlaSerLeuArgLeuArgSerAla    539
```

Fig.5

```
CATGGAGACGGCAGGCTGCTTTGTGGAGCTACCCTTCTGAGTAGCTGCTGGGTCCTGACA   1921
HisGlyAspGlyArgLeuLeuCysGlyAlaThrLeuLeuSerSerCysTrpValLeuThr    559

GCTGCACACTGCTTCAAAAGGTACGGAAACAACTCGAGGAGCTATGCAGTTCGAGTTGGG   1981
AlaAlaHisCysPheLysArgTyrGlyAsnAsnSerArgSerTyrAlaValArgValGly    579

GATTATCATACTCTGGTACCAGAGGAGTTTGAACAAGAAATAGGGGTTCAACAGATTGTG   2041
AspTyrHisThrLeuValProGluGluPheGluGlnGluIleGlyValGlnGlnIleVal    599

ATTCACAGGAACTACAGGCCAGACAGAAGCGACTATGACATTGCCCTGGTTAGATTGCAA   2101
IleHisArgAsnTyrArgProAspArgSerAspTyrAspIleAlaLeuValArgLeuGln    619

GGACCAGGGGAGCAATGTGCCAGACTAAGCACCCACGTTTTGCCAGCCTGTTTACCTCTA   2161
GlyProGlyGluGlnCysAlaArgLeuSerThrHisValLeuProAlaCysLeuProLeu    639

TGGAGAGAGAGGCCACAGAAAACAGCCTCCAACTGTCACATAACAGGATGGGGAGACACA   2221
TrpArgGluArgProGlnLysThrAlaSerAsnCysHisIleThrGlyTrpGlyAspThr    659

GGTCGTGCCTACTCAAGAACTCTACAACAAGCTGCTGTGCCTCTGTTACCCAAGAGGTTT   2281
GlyArgAlaTyrSerArgThrLeuGlnGlnAlaAlaValProLeuLeuProLysArgPhe    679
```

Fig.6

```
TGTAAAGAGAGGTACAAGGGACTATTTACTGGGAGAATGCTCTGTGCTGGGAACCTCCAA  2341
CysLysGluArgTyrLysGlyLeuPheThrGlyArgMetLeuCysAlaGlyAsnLeuGln   699

GAAGACAACCGTGTGGACAGCTGCCAGGGAGACAGTGGAGGACCACTCATGTGTGAAAAG  2401
GluAspAsnArgValAspSerCysGlnGlyAspSerGlyGlyProLeuMetCysGluLys   719

CCTGATGAGTCCTGGGTTGTGTATGGGGTGACTTCCTGGGGGTATGGATGTGGAGTCAAA  2461
ProAspGluSerTrpValValTyrGlyValThrSerTrpGlyTyrGlyCysGlyValLys   739

GACACTCCTGGAGTTTATACCAGAGTCCCCGCCTTTGTACCTTGGATAAAAAGTGTCACC  2521
AspThrProGlyValTyrThrArgValProAlaPheValProTrpIleLysSerValThr   759

AGTCTGTAACTTATGGAAAGCTCAAGAAAATAGTAAAACAGTAACCATTCAGTCTTCATA  2581
SerLeu***                                                      761

CTTGGCACCATGCCAGAAAAAAAAAAAAAAAAA                             2614
```

Fig.7

CCGACGACGCGTCCGCCGCCGCCTCTCCCGCGCTTCCCGCGCCCCCGCGGGCGCTCCCT 60
ProThrThrArgProProProProLeuProArgPheProArgProProArgAlaLeuPro 20

GCCCAGCGCCCGCACGCCCTCCAGGCCGGGCACACGCCCCGGCCGCACCCCTGGGGCTGC 120
AlaGlnArgProHisAlaLeuGlnAlaGlyHisThrProArgProHisProTrpGlyCys 40

CCCGCCGGCGAGCCATGGGTCAGCGTGACGGACTTCGGCGCCCCGTGTCTGCGGTGGGCG 180
ProAlaGlyGluProTrpValSerValThrAspPheGlyAlaProCysLeuArgTrpAla 60

GAGGTGCCACCCTTCCTGGAGCGGTCGCCCCCAGCGAGCTGGGCTCAGCTGCGAGGACAG 240
GluValProProPheLeuGluArgSerProProAlaSerTrpAlaGlnLeuArgGlyGln 80

CGCCACAACTTTTGTCGGAGCCCCGACGGCGCGGGCAGACCCTGGTGTTTCTACGGAGAC 300
ArgHisAsnPheCysArgSerProAspGlyAlaGlyArgProTrpCysPheTyrGlyAsp 100

GCCCGTGGCAAGGTGGACTGGGGCTACTGCGACTGCAGACACGGATCAGTACGACTTCGT 360
AlaArgGlyLysValAspTrpGlyTyrCysAspCysArgHisGlySerValArgLeuArg 120

GGCGGCAAAAATGAGTTTGAAGGCACAGTGGAAGTATATGCAAGTGGAGTTTGGGGCACT 420
GlyGlyLysAsnGluPheGluGlyThrValGluValTyrAlaSerGlyValTrpGlyThr 140

Fig.8

```
GTCTGTAGCAGCCACTGGGATGATTCTGATGCATCAGTCATTTGTCACCAGCTGCAGCTG   480
ValCysSerSerHisTrpAspAspSerAspAlaSerValIleCysHisGlnLeuGlnLeu   160

GGAGGAAAAGGAATAGCAAAACAAACCCCGTTTTCTGGACTGGGCCTTATTCCCATTTAT   540
GlyGlyLysGlyIleAlaLysGlnThrProPheSerGlyLeuGlyLeuIleProIleTyr   180

TGGAGCAATGTCCGTTGCCGAGGAGATGAAGAAAATATACTGCTTTGTGAAAAAGACATC   600
TrpSerAsnValArgCysArgGlyAspGluGluAsnIleLeuLeuCysGluLysAspIle   200

TGGCAGGGTGGGGTGTGTCCTCAGAAGATGGCAGCTGCTGTCACGTGTAGCTTTTCCCAT   660
TrpGlnGlyGlyValCysProGlnLysMetAlaAlaAlaValThrCysSerPheSerHis   220

GGCCCAACGTTCCCCATCATTCGCCTTGCTGGAGGCAGCAGTGTGCATGAAGGCCGGGTG   720
GlyProThrPheProIleIleArgLeuAlaGlyGlySerSerValHisGluGlyArgVal   240

GAGCTCTACCATGCTGGCCAGTGGGGAACCGTTTGTGATGACCAATGGGATGATGCCGAT   780
GluLeuTyrHisAlaGlyGlnTrpGlyThrValCysAspAspGlnTrpAspAspAlaAsp   260

GCAGAAGTGATCTGCAGGCAGCTGGGCCTCAGTGGCATTGCCAAAGCATGGCATCAGGCA   840
AlaGluValIleCysArgGlnLeuGlyLeuSerGlyIleAlaLysAlaTrpHisGlnAla   280
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Fig.9

```
TATTTTGGGGAAGGGTCTGGCCCAGTTATGTTGGATGAAGTACGCTGCACTGGGAATGAG   900
TyrPheGlyGluGlySerGlyProValMetLeuAspGluValArgCysThrGlyAsnGlu   300

CTTTCAATTGAGCAGTGTCCAAAGAGCTCCTGGGGAGAGCATAACTGTGGCCATAAAGAA   960
LeuSerIleGluGlnCysProLysSerSerTrpGlyGluHisAsnCysGlyHisLysGlu   320

GATGCTGGAGTGTCCTGTACCCCTCTAACAGATGGGGTCATCAGACTTGCAGGTGGGAAA  1020
AspAlaGlyValSerCysThrProLeuThrAspGlyValIleArgLeuAlaGlyGlyLys   340

GGCAGCCATGAGGGTCGCTTGGAGGTATATTACAGAGGCCAGTGGGGAACTGTCTGTGAT  1080
GlySerHisGluGlyArgLeuGluValTyrTyrArgGlyGlnTrpGlyThrValCysAsp   360

GATGGCTGGACTGAGCTGAATACATACGTGGTTTGTCGACAGTTGGGATTTAAATATGGT  1140
AspGlyTrpThrGluLeuAsnThrTyrValValCysArgGlnLeuGlyPheLysTyrGly   380

AAACAAGCATCTGCCAACCATTTTGAAGAAAGCACAGGGCCCATATGGTTGGATGACGTC  1200
LysGlnAlaSerAlaAsnHisPheGluGluSerThrGlyProIleTrpLeuAspAspVal   400

AGCTGCTCAGGAAAGGAAACCAGATTTCTTCAGTGTTCCAGGCGACAGTGGGGAAGGCAT  1260
SerCysSerGlyLysGluThrArgPheLeuGlnCysSerArgArgGlnTrpGlyArgHis   420
```

Fig.10

GACTGCAGCCACCGCGAAGATGTTAGCATTGCCTGCTACCCTGGCGGCGAGGGACACAGG 1320
AspCysSerHisArgGluAspValSerIleAlaCysTyrProGlyGlyGluGlyHisArg 440

CTCTCTCTGGGTTTTCCTGTCAGACTGATGGATGGAGAAAATAAGAAAGAAGGACGAGTG 1380
LeuSerLeuGlyPheProValArgLeuMetAspGlyGluAsnLysLysGluGlyArgVal 460

GAGGTTTTTATCAATGGCCAGTGGGGAACAATCTGTGATGATGGATGGACTGATAAGGAT 1440
GluValPheIleAsnGlyGlnTrpGlyThrIleCysAspAspGlyTrpThrAspLysAsp 480

GCAGCTGTGATCTGTCGTCAGCTTGGCTACAAGGGTCCTGCCAGAGCAAGAACCATGGCT 1500
AlaAlaValIleCysArgGlnLeuGlyTyrLysGlyProAlaArgAlaArgThrMetAla 500

TACTTTGGAGAAGGAAAAGGACCCATCCATGTGGATAATGTGAAGTGCACAGGAAATGAG 1560
TyrPheGlyGluGlyLysGlyProIleHisValAspAsnValLysCysThrGlyAsnGlu 520

AGGTCCTTGGCTGACTGTATCAAGCAAGATATTGGAAGACACAACTGCCGCCACAGTGAA 1620
ArgSerLeuAlaAspCysIleLysGlnAspIleGlyArgHisAsnCysArgHisSerGlu 540

GATGCAGGAGTTATTTGTGATTATTTTGGCAAGAAGGCCTCAGGTAACAGTAATAAAGAG 1680
AspAlaGlyValIleCysAspTyrPheGlyLysLysAlaSerGlyAsnSerAsnLysGlu 560

Fig.11

```
TCCCTCTCATCTGTTTGTGGCTTGAGATTACTGCACCGTCGGCAGAAGCGGATCATTGGT   1740
SerLeuSerSerValCysGlyLeuArgLeuLeuHisArgArgGlnLysArgIleIleGly    580

GGGAAAAATTCTTTAAGGGGTGGTTGGCCTTGGCAGGTTTCCCTCCGGCTGAAGTCATCC   1800
GlyLysAsnSerLeuArgGlyGlyTrpProTrpGlnValSerLeuArgLeuLysSerSer    600

CATGGAGATGGCAGGCTCCTCTGCGGGGCTACGCTCCTGAGTAGCTGCTGGGTCCTCACA   1860
HisGlyAspGlyArgLeuLeuCysGlyAlaThrLeuLeuSerSerCysTrpValLeuThr    620

GCAGCACACTGTTTCAAGAGGTATGGCAACAGCACTAGGAGCTATGCTGTTAGGGTTGGA   1920
AlaAlaHisCysPheLysArgTyrGlyAsnSerThrArgSerTyrAlaValArgValGly    640

GATTATCATACTCTGGTACCAGAGGAGTTTGAGGAAGAAATTGGAGTTCAACAGATTGTG   1980
AspTyrHisThrLeuValProGluGluPheGluGluGluIleGlyValGlnGlnIleVal    660

ATTCATCGGGAGTATCGACCCGACCGCAGTGATTATGACATAGCCCTGGTTAGATTACAA   2040
IleHisArgGluTyrArgProAspArgSerAspTyrAspIleAlaLeuValArgLeuGln    680

GGACCAGAAGAGCAATGTGCCAGATTCAGCAGCCATGTTTTGCCAGCCTGTTTACCACTC   2100
GlyProGluGluGlnCysAlaArgPheSerSerHisValLeuProAlaCysLeuProLeu    700
```

Fig.12

```
TGGAGAGAGAGGCCACAGAAAACAGCATCCAACTGTTACATAACAGGATGGGGTGACACA  2160
TrpArgGluArgProGlnLysThrAlaSerAsnCysTyrIleThrGlyTrpGlyAspThr   720

GGACGAGCCTATTCAAGAACACTACAACAAGCAGCCATTCCCTTACTTCCTAAAAGGTTT  2220
GlyArgAlaTyrSerArgThrLeuGlnGlnAlaAlaIleProLeuLeuProLysArgPhe   740

TGTGAAGAACGTTATAAGGGTCGGTTTACAGGGAGAATGCTTTGTGCTGGAAACCTCCAT  2280
CysGluGluArgTyrLysGlyArgPheThrGlyArgMetLeuCysAlaGlyAsnLeuHis   760

GAACACAAACGCGTGGACAGCTGCCAGGGAGACAGCGGAGGACCACTCATGTGTGAACGG  2340
GluHisLysArgValAspSerCysGlnGlyAspSerGlyGlyProLeuMetCysGluArg   780

CCCGGAGAGAGCTGGGTGGTGTATGGGGTGACCTCCTGGGGGTATGGCTGTGGAGTCAAG  2400
ProGlyGluSerTrpValValTyrGlyValThrSerTrpGlyTyrGlyCysGlyValLys   800

GATTCTCCTGGTGTTTATACCAAAGTCTCAGCCTTTGTACCTTGGATAAAAAGTGTCACC  2460
AspSerProGlyValTyrThrLysValSerAlaPheValProTrpIleLysSerValThr   820

AAACTGTAATTCTTCATGGAAACTTCAAAGCAGCATTTAAACAAATGGAAAACTTTGAAC  2520
LysLeu***                                                      822

CCCCACTATTAGCACTCAGCAGAGATGACAACAAACGGCAAG                    2562
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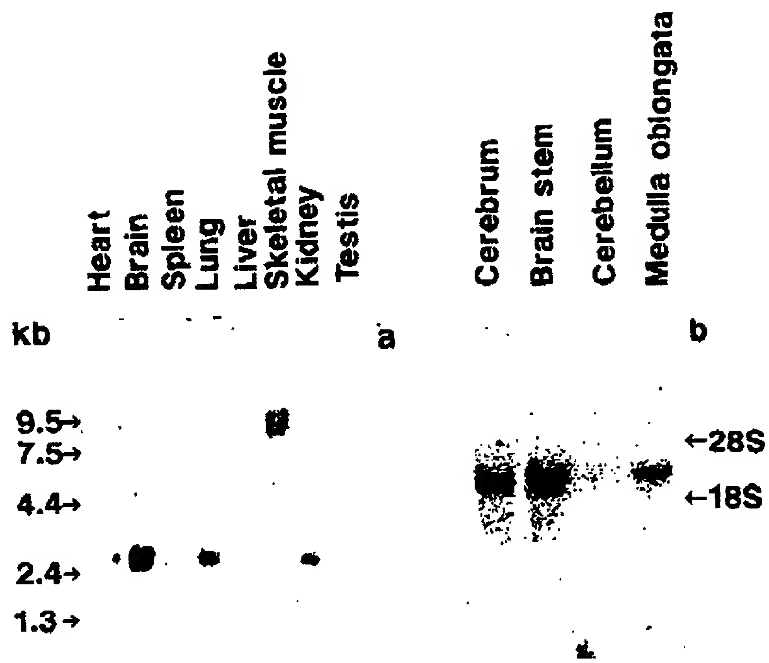
Fig.13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Spleen
Lung
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Skeletal muscle
Kidney
Testis
Cerebrum
Brain stem
Cerebellum
Medulla oblongata
kb
a
b
9.5→
7.5→
4.4→
2.4→
1.3→
←28S
←18S

Declaration and Power of Attorney For Patent Application

特許出願宣言書及び委任状

Japanese Language Declaration

日本語宣言書

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______________________	NOVEL SERINE PROTEASE
______________________	______________________
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Prior Foreign Application(s)
外国での先行出願

(Number) (番号)	(Country) (国名)	(Day/Month/Year Filed) (出願年月日)	Priority Not Claimed 優先権主張なし
9-213969(Pat. Appln.)	Japan	24/July/1997	☐
			☐

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第六共同発明者	Full name of sixth joint inventor, if any
第六共同発明者 日付	Sixth inventor's signature Date
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国　籍	Citizenship
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tct gta gtg gcc cgc gct gat ccg gtc tcg cgc tct ccc ctt cac cgc 337
Ser Val Val Ala Arg Ala Asp Pro Val Ser Arg Ser Pro Leu His Arg
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ccg cat ccg tcc cca ccg cgt tcc caa cac gcg cac tac ctt ccc agc 385
Pro His Pro Ser Pro Pro Arg Ser Gln His Ala His Tyr Leu Pro Ser
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tcg cgg cgg cca ccc agg acc ccg cgc ttc ccg ctc ccg ctg cgg atc 433
Ser Arg Arg Pro Pro Arg Thr Pro Arg Phe Pro Leu Pro Leu Arg Ile
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ccc gct gcc cag cgc ccg cag gtc ctc agc acc ggg cac acg ccc ccg 481
Pro Ala Ala Gln Arg Pro Gln Val Leu Ser Thr Gly His Thr Pro Pro
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acg att cca cgc cgc tgc ggg gca gga gag tcg tgg ggc aat gcc acc 529
Thr Ile Pro Arg Arg Cys Gly Ala Gly Glu Ser Trp Gly Asn Ala Thr
80 85 90 95

aac ctc ggc gtc ccg tgt cta cac tgg gac gag gtg ccg ccc ttc ctg 577
Asn Leu Gly Val Pro Cys Leu His Trp Asp Glu Val Pro Pro Phe Leu
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gag cgg tcg ccc ccg gcc agt tgg gct gag ctg cga ggg cag ccg cac 625
Glu Arg Ser Pro Pro Ala Ser Trp Ala Glu Leu Arg Gly Gln Pro His
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aac ttc tgc cgg agc ccg gat ggc tcg ggc aga cct tgg tgc ttc tat 673
Asn Phe Cys Arg Ser Pro Asp Gly Ser Gly Arg Pro Trp Cys Phe Tyr
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cgg aat gcc cag ggc aaa gta gac tgg ggc tac tgc gat tgt ggt caa 721
Arg Asn Ala Gln Gly Lys Val Asp Trp Gly Tyr Cys Asp Cys Gly Gln
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ggc ccg gcg ttg ccc gtc att cgc ctt gtt ggt ggg aac agt ggg cat 769
Gly Pro Ala Leu Pro Val Ile Arg Leu Val Gly Gly Asn Ser Gly His
160 165 170 175

gaa ggt cga gtg gag ctg tac cac gct ggc cag tgg ggg acc atc tgt 817
Glu Gly Arg Val Glu Leu Tyr His Ala Gly Gln Trp Gly Thr Ile Cys
180 185 190

gac gac caa tgg gac aat gca gac gca gac gtc atc tgt agg cag ctg 865
Asp Asp Gln Trp Asp Asn Ala Asp Ala Asp Val Ile Cys Arg Gln Leu
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Gly Ser Gly Pro Ile Leu Leu Asp Glu Val Arg Cys Thr Gly Asn Glu
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ctg tca att gag caa tgt cca aag agt tcc tgg ggc gaa cat aac tgt     1009
Leu Ser Ile Glu Gln Cys Pro Lys Ser Ser Trp Gly Glu His Asn Cys
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ggc cat aaa gaa gat gct gga gtg tct tgt gtt cct cta aca gat ggt     1057
Gly His Lys Glu Asp Ala Gly Val Ser Cys Val Pro Leu Thr Asp Gly
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gtc atc aga ctg gca gga gga aaa agt acc cat gaa ggt cgc ctg gag     1105
Val Ile Arg Leu Ala Gly Gly Lys Ser Thr His Glu Gly Arg Leu Glu
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gtc tac tac aag ggg cag tgg ggg aca gtc tgt gat gat ggc tgg act     1153
Val Tyr Tyr Lys Gly Gln Trp Gly Thr Val Cys Asp Asp Gly Trp Thr
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gag atg aac aca tac gtg gct tgt cga ctg ctg gga ttt aaa tac ggc     1201
Glu Met Asn Thr Tyr Val Ala Cys Arg Leu Leu Gly Phe Lys Tyr Gly
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aaa cag tcc tct gtg aac cat ttt gat ggc agc aac agg ccc ata tgg     1249
Lys Gln Ser Ser Val Asn His Phe Asp Gly Ser Asn Arg Pro Ile Trp
320                 325                 330                 335

ctg gat gac gtc agc tgc tca gga aaa gaa gtc agc ttc att cag tgt     1297
Leu Asp Asp Val Ser Cys Ser Gly Lys Glu Val Ser Phe Ile Gln Cys
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tcc agg aga cag tgg gga agg cat gac tgc agc cat aga gaa gat gtg     1345
Ser Arg Arg Gln Trp Gly Arg His Asp Cys Ser His Arg Glu Asp Val
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ggc ctc acc tgc tat cct gac agc gat gga cat agg ctt tct cca ggt     1393
Gly Leu Thr Cys Tyr Pro Asp Ser Asp Gly His Arg Leu Ser Pro Gly
        370                 375                 380

ttt ccc atc aga cta gtg gat gga gag aat aag aag gaa gga cga gtg     1441
Phe Pro Ile Arg Leu Val Asp Gly Glu Asn Lys Lys Glu Gly Arg Val
    385                 390                 395
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Glu Val Phe Val Asn Gly Gln Trp Gly Thr Ile Cys Asp Asp Gly Trp
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acc gat aag cat gca gct gtg atc tgc cgg cag ctt ggc tat aag ggt 1537
Thr Asp Lys His Ala Ala Val Ile Cys Arg Gln Leu Gly Tyr Lys Gly
420 425 430

cct gcc aga gca agg act atg gct tat ttt ggg gaa gga aaa ggc ccc 1585
Pro Ala Arg Ala Arg Thr Met Ala Tyr Phe Gly Glu Gly Lys Gly Pro
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atc cac atg gat aat gtg aag tgc aca gga aat gag aag gcc ctg gct 1633
Ile His Met Asp Asn Val Lys Cys Thr Gly Asn Glu Lys Ala Leu Ala
450 455 460

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Asp Cys Val Lys Gln Asp Ile Gly Arg His Asn Cys Arg His Ser Glu
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gat gca gga gtc atc tgt gac tat tta gag aag aaa gca tca agt agt 1729
Asp Ala Gly Val Ile Cys Asp Tyr Leu Glu Lys Lys Ala Ser Ser Ser
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ggt aat aaa gag atg ctc tca tct gga tgt gga ctg agg tta ctg cac 1777
Gly Asn Lys Glu Met Leu Ser Ser Gly Cys Gly Leu Arg Leu Leu His
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Arg Arg Gln Lys Arg Ile Ile Gly Gly Asn Asn Ser Leu Arg Gly Ala
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Trp Pro Trp Gln Ala Ser Leu Arg Leu Arg Ser Ala His Gly Asp Gly
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Arg Leu Leu Cys Gly Ala Thr Leu Leu Ser Ser Cys Trp Val Leu Thr
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Ala Ala His Cys Phe Lys Arg Tyr Gly Asn Asn Ser Arg Ser Tyr Ala
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gtt cga gtt ggg gat tat cat act ctg gta cca gag gag ttt gaa caa 2017
Val Arg Val Gly Asp Tyr His Thr Leu Val Pro Glu Glu Phe Glu Gln
580 585 590

gaa ata ggg gtt caa cag att gtg att cac agg aac tac agg cca gac . 2065
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Arg Ser Asp Tyr Asp Ile Ala Leu Val Arg Leu Gln Gly Pro Gly Glu
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Gln Cys Ala Arg Leu Ser Thr His Val Leu Pro Ala Cys Leu Pro Leu
625 630 635

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Trp Arg Glu Arg Pro Gln Lys Thr Ala Ser Asn Cys His Ile Thr Gly
640 645 650 655

tgg gga gac aca ggt cgt gcc tac tca aga act cta caa caa gct gct 2257
Trp Gly Asp Thr Gly Arg Ala Tyr Ser Arg Thr Leu Gln Gln Ala Ala
660 665 670

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Val Pro Leu Leu Pro Lys Arg Phe Cys Lys Glu Arg Tyr Lys Gly Leu
675 680 685

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Phe Thr Gly Arg Met Leu Cys Ala Gly Asn Leu Gln Glu Asp Asn Arg
690 695 700

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Val Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Cys Glu Lys
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cct gat gag tcc tgg gtt gtg tat ggg gtg act tcc tgg ggg tat gga 2449
Pro Asp Glu Ser Trp Val Val Tyr Gly Val Thr Ser Trp Gly Tyr Gly
720 725 730 735

tgt gga gtc aaa gac act cct gga gtt tat acc aga gtc ccc gcc ttt 2497
Cys Gly Val Lys Asp Thr Pro Gly Val Tyr Thr Arg Val Pro Ala Phe
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gta cct tgg ata aaa agt gtc acc agt ctg taacttatgg aaagctcaag 2547
Val Pro Trp Ile Lys Ser Val Thr Ser Leu
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35 40 45

Arg Arg Pro Pro Arg Thr Pro Arg Phe Pro Leu Pro Leu Arg Ile Pro
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Ala Ala Gln Arg Pro Gln Val Leu Ser Thr Gly His Thr Pro Pro Thr
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Ile Pro Arg Arg Cys Gly Ala Gly Glu Ser Trp Gly Asn Ala Thr Asn
85 90 95

Leu Gly Val Pro Cys Leu His Trp Asp Glu Val Pro Pro Phe Leu Glu
100 105 110

Arg Ser Pro Pro Ala Ser Trp Ala Glu Leu Arg Gly Gln Pro His Asn
115 120 125

Phe Cys Arg Ser Pro Asp Gly Ser Gly Arg Pro Trp Cys Phe Tyr Arg
130 135 140

Asn Ala Gln Gly Lys Val Asp Trp Gly Tyr Cys Asp Cys Gly Gln Gly
145 150 155 160

Pro Ala Leu Pro Val Ile Arg Leu Val Gly Gly Asn Ser Gly His Glu
165 170 175

Gly Arg Val Glu Leu Tyr His Ala Gly Gln Trp Gly Thr Ile Cys Asp
180 185 190

Asp Gln Trp Asp Asn Ala Asp Ala Asp Val Ile Cys Arg Gln Leu Gly
195 200 205

Leu Ser Gly Ile Ala Lys Ala Trp His Gln Ala His Phe Gly Glu Gly
210 215 220

Ser Gly Pro Ile Leu Leu Asp Glu Val Arg Cys Thr Gly Asn Glu Leu
225 230 235 240

Ser Ile Glu Gln Cys Pro Lys Ser Ser Trp Gly Glu His Asn Cys Gly
245 250 255

His Lys Glu Asp Ala Gly Val Ser Cys Val Pro Leu Thr Asp Gly Val
260 265 270

Ile Arg Leu Ala Gly Gly Lys Ser Thr His Glu Gly Arg Leu Glu Val
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Tyr Tyr Lys Gly Gln Trp Gly Thr Val Cys Asp Asp Gly Trp Thr Glu
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Gln Ser Ser Val Asn His Phe Asp Gly Ser Asn Arg Pro Ile Trp Leu
325 330 335

Asp Asp Val Ser Cys Ser Gly Lys Glu Val Ser Phe Ile Gln Cys Ser
340 345 350

Arg Arg Gln Trp Gly Arg His Asp Cys Ser His Arg Glu Asp Val Gly
355 360 365

Leu Thr Cys Tyr Pro Asp Ser Asp Gly His Arg Leu Ser Pro Gly Phe
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Pro Ile Arg Leu Val Asp Gly Glu Asn Lys Lys Glu Gly Arg Val Glu
385 390 395 400

Val Phe Val Asn Gly Gln Trp Gly Thr Ile Cys Asp Asp Gly Trp Thr
405 410 415

Asp Lys His Ala Ala Val Ile Cys Arg Gln Leu Gly Tyr Lys Gly Pro
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Ala Arg Ala Arg Thr Met Ala Tyr Phe Gly Glu Gly Lys Gly Pro Ile
435 440 445

His Met Asp Asn Val Lys Cys Thr Gly Asn Glu Lys Ala Leu Ala Asp
450 455 460

Cys Val Lys Gln Asp Ile Gly Arg His Asn Cys Arg His Ser Glu Asp
465 470 475 480

Ala Gly Val Ile Cys Asp Tyr Leu Glu Lys Lys Ala Ser Ser Ser Gly
485 490 495

Asn Lys Glu Met Leu Ser Ser Gly Cys Gly Leu Arg Leu Leu His Arg
500 505 510

Arg Gln Lys Arg Ile Ile Gly Gly Asn Asn Ser Leu Arg Gly Ala Trp
515 520 525

Pro Trp Gln Ala Ser Leu Arg Leu Arg Ser Ala His Gly Asp Gly Arg
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Ala His Cys Phe Lys Arg Tyr Gly Asn Asn Ser Arg Ser Tyr Ala Val
565 570 575

Arg Val Gly Asp Tyr His Thr Leu Val Pro Glu Glu Phe Glu Gln Glu
580 585 590

Ile Gly Val Gln Gln Ile Val Ile His Arg Asn Tyr Arg Pro Asp Arg
595 600 605

Ser Asp Tyr Asp Ile Ala Leu Val Arg Leu Gln Gly Pro Gly Glu Gln
610 615 620

Cys Ala Arg Leu Ser Thr His Val Leu Pro Ala Cys Leu Pro Leu Trp
625 630 635 640

Arg Glu Arg Pro Gln Lys Thr Ala Ser Asn Cys His Ile Thr Gly Trp
645 650 655

Gly Asp Thr Gly Arg Ala Tyr Ser Arg Thr Leu Gln Gln Ala Ala Val
660 665 670

Pro Leu Leu Pro Lys Arg Phe Cys Lys Glu Arg Tyr Lys Gly Leu Phe
675 680 685.

Thr Gly Arg Met Leu Cys Ala Gly Asn Leu Gln Glu Asp Asn Arg Val
690 695 700

Asp Ser Cys Gln Gly Asp Ser Gly Gly Pro Leu Met Cys Glu Lys Pro
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Asp Glu Ser Trp Val Val Tyr Gly Val Thr Ser Trp Gly Tyr Gly Cys
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Arg Ala Leu Pro Ala Gln Arg Pro His Ala Leu Gln Ala Gly His Thr

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Pro Arg Pro His Pro Trp Gly Cys Pro Ala Gly Glu Pro Trp Val Ser

35 40 45

gtg acg gac ttc ggc gcc ccg tgt ctg cgg tgg gcg gag gtg cca ccc 192

Val Thr Asp Phe Gly Ala Pro Cys Leu Arg Trp Ala Glu Val Pro Pro

50 55 60

ttc ctg gag cgg tcg ccc cca gcg agc tgg gct cag ctg cga gga cag 240

Phe Leu Glu Arg Ser Pro Pro Ala Ser Trp Ala Gln Leu Arg Gly Gln

65 70 75 80

cgc cac aac ttt tgt cgg agc ccc gac ggc gcg ggc aga ccc tgg tgt 288

Arg His Asn Phe Cys Arg Ser Pro Asp Gly Ala Gly Arg Pro Trp Cys

85 90 95

ttc tac gga gac gcc cgt ggc aag gtg gac tgg ggc tac tgc gac tgc 336

Phe Tyr Gly Asp Ala Arg Gly Lys Val Asp Trp Gly Tyr Cys Asp Cys

100 105 110

aga cac gga tca gta cga ctt cgt ggc ggc aaa aat gag ttt gaa ggc 384

Arg His Gly Ser Val Arg Leu Arg Gly Gly Lys Asn Glu Phe Glu Gly

115 120 125

aca gtg gaa gta tat gca agt gga gtt tgg ggc act gtc tgt agc agc 432

Thr Val Glu Val Tyr Ala Ser Gly Val Trp Gly Thr Val Cys Ser Ser

130 135 140

cac tgg gat gat tct gat gca tca gtc att tgt cac cag ctg cag ctg 480

His Trp Asp Asp Ser Asp Ala Ser Val Ile Cys His Gln Leu Gln Leu

145 150 155 160

gga gga aaa gga ata gca aaa caa acc ccg ttt tct gga ctg ggc ctt 528

Gly Gly Lys Gly Ile Ala Lys Gln Thr Pro Phe Ser Gly Leu Gly Leu

165 170 175

att ccc att tat tgg agc aat gtc cgt tgc cga gga gat gaa gaa aat 576

Ile Pro Ile Tyr Trp Ser Asn Val Arg Cys Arg Gly Asp Glu Glu Asn

180 185 190

ata ctg ctt tgt gaa aaa gac atc tgg cag ggt ggg gtg tgt cct cag 624

Ile Leu Leu Cys Glu Lys Asp Ile Trp Gln Gly Gly Val Cys Pro Gln

195 200 205

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aag atg gca gct gct gtc acg tgt agc ttt tcc cat ggc cca acg ttc      672
Lys Met Ala Ala Ala Val Thr Cys Ser Phe Ser His Gly Pro Thr Phe
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Pro Ile Ile Arg Leu Ala Gly Gly Ser Ser Val His Glu Gly Arg Val
225                 230                 235                 240
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Glu Leu Tyr His Ala Gly Gln Trp Gly Thr Val Cys Asp Asp Gln Trp
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Asp Asp Ala Asp Ala Glu Val Ile Cys Arg Gln Leu Gly Leu Ser Gly
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Ile Ala Lys Ala Trp His Gln Ala Tyr Phe Gly Glu Gly Ser Gly Pro
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Val Met Leu Asp Glu Val Arg Cys Thr Gly Asn Glu Leu Ser Ile Glu
    290                 295                 300
cag tgt cca aag agc tcc tgg gga gag cat aac tgt ggc cat aaa gaa      960
Gln Cys Pro Lys Ser Ser Trp Gly Glu His Asn Cys Gly His Lys Glu
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Asp Ala Gly Val Ser Cys Thr Pro Leu Thr Asp Gly Val Ile Arg Leu
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Ala Gly Gly Lys Gly Ser His Glu Gly Arg Leu Glu Val Tyr Tyr Arg
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Gly Gln Trp Gly Thr Val Cys Asp Asp Gly Trp Thr Glu Leu Asn Thr
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tac gtg gtt tgt cga cag ttg gga ttt aaa tat ggt aaa caa gca tct     1152
Tyr Val Val Cys Arg Gln Leu Gly Phe Lys Tyr Gly Lys Gln Ala Ser
    370                 375                 380
gcc aac cat ttt gaa gaa agc aca ggg ccc ata tgg ttg gat gac gtc     1200
Ala Asn His Phe Glu Glu Ser Thr Gly Pro Ile Trp Leu Asp Asp Val
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Trp Gly Arg His Asp Cys Ser His Arg Glu Asp Val Ser Ile Ala Cys
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Tyr Pro Gly Gly Glu Gly His Arg Leu Ser Leu Gly Phe Pro Val Arg
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Leu Met Asp Gly Glu Asn Lys Lys Glu Gly Arg Val Glu Val Phe Ile
450 455 460

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Asn Gly Gln Trp Gly Thr Ile Cys Asp Asp Gly Trp Thr Asp Lys Asp
465 470 475 480

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Ala Ala Val Ile Cys Arg Gln Leu Gly Tyr Lys Gly Pro Ala Arg Ala
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Arg Thr Met Ala Tyr Phe Gly Glu Gly Lys Gly Pro Ile His Val Asp
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Asn Val Lys Cys Thr Gly Asn Glu Arg Ser Leu Ala Asp Cys Ile Lys
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Gln Asp Ile Gly Arg His Asn Cys Arg His Ser Glu Asp Ala Gly Val
530 535 540

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Ile Cys Asp Tyr Phe Gly Lys Lys Ala Ser Gly Asn Ser Asn Lys Glu
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Ser Leu Ser Ser Val Cys Gly Leu Arg Leu Leu His Arg Arg Gln Lys
565 570 575

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Arg Ile Ile Gly Gly Lys Asn Ser Leu Arg Gly Gly Trp Pro Trp Gln
580 585 590

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Val Ser Leu Arg Leu Lys Ser Ser His Gly Asp Gly Arg Leu Leu Cys
595 600 605

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Gly Ala Thr Leu Leu Ser Ser Cys Trp Val Leu Thr Ala Ala His Cys
610 615 620

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625 630 635 640

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Asp Tyr His Thr Leu Val Pro Glu Glu Phe Glu Glu Glu Ile Gly Val
645 650 655

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Gln Gln Ile Val Ile His Arg Glu Tyr Arg Pro Asp Arg Ser Asp Tyr
660 665 670

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675 680 685

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Phe Ser Ser His Val Leu Pro Ala Cys Leu Pro Leu Trp Arg Glu Arg
690 695 700

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725 730 735

cct aaa agg ttt tgt gaa gaa cgt tat aag ggt cgg ttt aca ggg aga 2256
Pro Lys Arg Phe Cys Glu Glu Arg Tyr Lys Gly Arg Phe Thr Gly Arg
740 745 750

atg ctt tgt gct gga aac ctc cat gaa cac aaa cgc gtg gac agc tgc 2304
Met Leu Cys Ala Gly Asn Leu His Glu His Lys Arg Val Asp Ser Cys
755 760 765

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Gln Gly Asp Ser Gly Gly Pro Leu Met Cys Glu Arg Pro Gly Glu Ser
770 775 780

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Trp Val Val Tyr Gly Val Thr Ser Trp Gly Tyr Gly Cys Gly Val Lys

785 790 795 800

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Asp Ser Pro Gly Val Tyr Thr Lys Val Ser Ala Phe Val Pro Trp Ile

805 810 815

aaa agt gtc acc aaa ctg taattcttca tggaaacttc aaagcagcat 2496

Lys Ser Val Thr Lys Leu

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35 40 45

Val Thr Asp Phe Gly Ala Pro Cys Leu Arg Trp Ala Glu Val Pro Pro

50 55 60

Phe Leu Glu Arg Ser Pro Pro Ala Ser Trp Ala Gln Leu Arg Gly Gln

65 70 75 80

Arg His Asn Phe Cys Arg Ser Pro Asp Gly Ala Gly Arg Pro Trp Cys

85 90 95

Phe Tyr Gly Asp Ala Arg Gly Lys Val Asp Trp Gly Tyr Cys Asp Cys

100 105 110

Arg His Gly Ser Val Arg Leu Arg Gly Gly Lys Asn Glu Phe Glu Gly

115 120 125

Thr Val Glu Val Tyr Ala Ser Gly Val Trp Gly Thr Val Cys Ser Ser

130 135 140

His Trp Asp Asp Ser Asp Ala Ser Val Ile Cys His Gln Leu Gln Leu
145 150 155 160
Gly Gly Lys Gly Ile Ala Lys Gln Thr Pro Phe Ser Gly Leu Gly Leu
165 170 175
Ile Pro Ile Tyr Trp Ser Asn Val Arg Cys Arg Gly Asp Glu Glu Asn
180 185 190
Ile Leu Leu Cys Glu Lys Asp Ile Trp Gln Gly Gly Val Cys Pro Gln
195 200 205
Lys Met Ala Ala Ala Val Thr Cys Ser Phe Ser His Gly Pro Thr Phe
210 215 220
Pro Ile Ile Arg Leu Ala Gly Gly Ser Ser Val His Glu Gly Arg Val
225 230 235 240
Glu Leu Tyr His Ala Gly Gln Trp Gly Thr Val Cys Asp Asp Gln Trp
245 250 255
Asp Asp Ala Asp Ala Glu Val Ile Cys Arg Gln Leu Gly Leu Ser Gly
260 265 270
Ile Ala Lys Ala Trp His Gln Ala Tyr Phe Gly Glu Gly Ser Gly Pro
275 280 285
Val Met Leu Asp Glu Val Arg Cys Thr Gly Asn Glu Leu Ser Ile Glu
290 295 300
Gln Cys Pro Lys Ser Ser Trp Gly Glu His Asn Cys Gly His Lys Glu
305 310 315 320
Asp Ala Gly Val Ser Cys Thr Pro Leu Thr Asp Gly Val Ile Arg Leu
325 330 335
Ala Gly Gly Lys Gly Ser His Glu Gly Arg Leu Glu Val Tyr Tyr Arg
340 345 350
Gly Gln Trp Gly Thr Val Cys Asp Asp Gly Trp Thr Glu Leu Asn Thr
355 360 365
Tyr Val Val Cys Arg Gln Leu Gly Phe Lys Tyr Gly Lys Gln Ala Ser
370 375 380
Ala Asn His Phe Glu Glu Ser Thr Gly Pro Ile Trp Leu Asp Asp Val
385 390 395 400
Ser Cys Ser Gly Lys Glu Thr Arg Phe Leu Gln Cys Ser Arg Arg Gln
405 410 415
Trp Gly Arg His Asp Cys Ser His Arg Glu Asp Val Ser Ile Ala Cys
420 425 430
Tyr Pro Gly Gly Glu Gly His Arg Leu Ser Leu Gly Phe Pro Val Arg
435 440 445

Leu Met Asp Gly Glu Asn Lys Lys Glu Gly Arg Val Glu Val Phe Ile
    450   455   460

Asn Gly Gln Trp Gly Thr Ile Cys Asp Asp Gly Trp Thr Asp Lys Asp
465   470   475   480

Ala Ala Val Ile Cys Arg Gln Leu Gly Tyr Lys Gly Pro Ala Arg Ala
                485   490   495

Arg Thr Met Ala Tyr Phe Gly Glu Gly Lys Gly Pro Ile His Val Asp
            500   505   510

Asn Val Lys Cys Thr Gly Asn Glu Arg Ser Leu Ala Asp Cys Ile Lys
        515   520   525

Gln Asp Ile Gly Arg His Asn Cys Arg His Ser Glu Asp Ala Gly Val
    530   535   540

Ile Cys Asp Tyr Phe Gly Lys Lys Ala Ser Gly Asn Ser Asn Lys Glu
545   550   555   560

Ser Leu Ser Ser Val Cys Gly Leu Arg Leu Leu His Arg Arg Gln Lys
                565   570   575

Arg Ile Ile Gly Gly Lys Asn Ser Leu Arg Gly Gly Trp Pro Trp Gln
            580   585   590

Val Ser Leu Arg Leu Lys Ser Ser His Gly Asp Gly Arg Leu Leu Cys
        595   600   605

Gly Ala Thr Leu Leu Ser Ser Cys Trp Val Leu Thr Ala Ala His Cys
    610   615   620

Phe Lys Arg Tyr Gly Asn Ser Thr Arg Ser Tyr Ala Val Arg Val Gly
625   630   635   640

Asp Tyr His Thr Leu Val Pro Glu Glu Phe Glu Glu Glu Ile Gly Val
                645   650   655

Gln Gln Ile Val Ile His Arg Glu Tyr Arg Pro Asp Arg Ser Asp Tyr
            660   665   670

Asp Ile Ala Leu Val Arg Leu Gln Gly Pro Glu Glu Gln Cys Ala Arg
        675   680   685

Phe Ser Ser His Val Leu Pro Ala Cys Leu Pro Leu Trp Arg Glu Arg
    690   695   700

Pro Gln Lys Thr Ala Ser Asn Cys Tyr Ile Thr Gly Trp Gly Asp Thr
705   710   715   720

Gly Arg Ala Tyr Ser Arg Thr Leu Gln Gln Ala Ala Ile Pro Leu Leu
                725   730   735

Pro Lys Arg Phe Cys Glu Glu Arg Tyr Lys Gly Arg Phe Thr Gly Arg
            740   745   750

Met Leu Cys Ala Gly Asn Leu His Glu His Lys Arg Val Asp Ser Cys
755 760 765

Gln Gly Asp Ser Gly Gly Pro Leu Met Cys Glu Arg Pro Gly Glu Ser
770 775 780

Trp Val Val Tyr Gly Val Thr Ser Trp Gly Tyr Gly Cys Gly Val Lys
785 790 795 800

Asp Ser Pro Gly Val Tyr Thr Lys Val Ser Ala Phe Val Pro Trp Ile
805 810 815

Lys Ser Val Thr Lys Leu
820